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PAPERS AND PROCEEDINGS OF THE ANNUAL MEETING

OF THE AMERICAN MOSQUITO CONTROL ASSOCIATION HELD JOINTLY WITH THE ANNUAL MEETING OF THE UTAH MOSQUITO ABATEMENT ASSOCIATION, SALT LAKE CITY, MARCH 24–27, 1952

Part 2 of two parts

In this issue *Mosquito News* completes the publication of the papers which constitute the *Proceedings* of the Annual Meeting held at Salt Lake City, March 24–27, 1952, with the exception of papers the manuscripts of which were not turned in to the editor or which have not yet reached the editorial office in final form. These remaining papers will be published as soon as possible after their receipt.

The publication of all the Proceedings papers in the two issues of *Mosquito News* which followed the annual meeting, resulting as it did in two exceptionally large numbers of the *News,* was made possible by the fact that the registration and other fees charged at the Salt Lake City meeting were turned in to the AMCA treasury for that purpose.

Special notice should be given to one of the titles listed in the program, namely, "Mosquito Culture Techniques—Revised," by Helen Louise Trembley. This paper will appear separately in the near future as Bulletin Number 3 of the American Mosquito Control Association. It will probably comprise at least sixty pages, and includes not only descriptions of original work and comprehensive synopses and analyses of contributions by other workers, but also a complete annotated bibliography of all the published work in the field of mosquito rearing.

D.L.C.

MOSQUITO CONTROL MOVES FORWARD

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Nobody loves a mosquito. Were the public fully aware of the part mosquitoes play in causing sickness, poverty, and starvation, its dislike would turn to a deep and persistent hatred of these pests. This knowledge might bring about an international "Kill the Mosquito Week," the issuance of an "Anti-Mosquito Stamp," the organization of "Down the Mosquito" clubs, and the provision of adequate funds for research on all angles of the mosquito problem and for control operations.

Is mosquito control really moving forward? Many people are skeptical as to whether we are making material progress against this common enemy of man.

There is reason for this skepticism. Some communities are discouraged over

the necessity to continue expending funds year after year without being completely freed of the pests. The appearance of strains of mosquitoes resistant to insecticides is presenting a serious obstacle to mosquito control in some areas. This situation, in turn, is leading into another problem—possible ill effects from unnecessarily heavy or frequent applications of insecticides. Such careless operations might contaminate pastures, forage, and gardens and harm fish, wildlife, beneficial insects, and even man.

These interferences with progress in mosquito control, though annoying and impeding the work to some extent, are not regarded as unsurmountable. This is evident from the reports of progress both here and abroad.

Yesterday we spoke of keeping malaria under control. Today we talk of eradication. Thus far 70 to 80 million people in various parts of the world have been given some measure of protection from malaria. Already malaria transmission has been stopped in Chile, Italy, and Cyprus and the mosquito vectors greatly reduced in numbers. Extensive eradication campaigns have been started also in many other countries. DDT is changing the outlook of the work despite the development of resistance of some mosquitoes to it.

Venezuela, under the competent leadership of A. Gabaldon has radically cut down the principal vector *Anopheles darlingi* Root and *A. albimanus* Wied., through drainage and house spraying. Gabaldon (4) has reported that 70 per cent of the houses in the malaria zone were sprayed in 1951 and an effort is being made to treat the remainder of the houses mostly in isolated areas. The malaria death rate has fallen from a median of 112 in 1941–45 to 9 in 1949. Gabaldon states "These facts represent the greatest change Venezuela has ever had in her health conditions."

Brazil started a vigorous anti-malaria campaign in 1947, when 200,000 houses were sprayed. This program has grown so that in 1950 more than 3 million houses were treated and the malaria parasite rate in the regularly sprayed areas has been reduced to a low point. Seventeen million of Brazil's people are exposed to malaria transmitted by 5 different species of *Anopheles*. Forests containing bromeliads in which *Anopheles bellator* D. & K. breed are being cut down. According to Pinotti (11) 80 per cent of the houses received residual sprays and the National Malaria Service had a staff of 8,000 employees in 1951. In the national program 3,000 tons of DDT are applied annually. Some of this insecticide is now being formulated in Brazil.

Programs are going forward in other Latin-American countries including Argentina, Peru, The Guianas, Bolivia, Colombia, Ecuador, and Mexico. During the first half of 1949 Argentina had only 2,785 cases of malaria as compared with 300,000 during the same part of previous years. In Peru industrial employers, the National Agrarian Society, and the National Social Security Fund all work together to keep the parasite rate down, according to Williams and Stowman (16).

British Guiana's malaria campaign, begun in 1947, is an outstanding example of what can be done with residual sprays alone. *Anopheles darlingi*, the vector, has been eradicated from the treated areas. The entire operation directed by Giglioli (6), cost only 20 cents per capita, and the annual saving to that country has been enormous, both in money and suffering.

Italy, which for years carried a heavy malaria burden is now essentially free from the disease. This accomplishment followed years of background information on the *Anopheles maculipennis* Meig. group built up by Professor Missiroli and his associates and his conception and execution of a five-year plan for control by DDT residual spraying which was started in 1946. Since 1948 not a single death from malaria has been reported (Russell, 12). In Sardinia the intensive program to eradicate *Anopheles labranchiae* Falleroni started by the Rockefeller Foundation in 1946 has wiped out malaria and all but eradicated the mosquito carrier.

The systematic use of residual sprays and larvicides was begun in the Karpas Peninsula of Cyprus in 1946, especially for the control of *Anopheles superpictus* Grassi, the principal carrier. The other half of the island was treated during the next two years. The cost per capita was 22 cents. In 1950 the Cyprus Government announced that malaria had been eradicated from this island.

In 1942 when Greece had 2 million cases of malaria, she consumed more than one-fifth of the world's output of quinine. In 1946, UNRRA started a control program in that country including residual spraying of buildings and aerial application of larvicides. G. D. Belios (2) reports that malaria cases in urban areas have dropped from a mean annual rate of 1,000,000 per year before World War II to 10,000 in 1948–49. In 1950 there were only 50,000 malaria cases in the entire country and now the disease has been practically eradicated. As a result of the antimalarial campaign Lividas (9) estimates the annual saving at $20 million.

Williams and Stowman (16) have summarized the results of antimalarial work in India and Ceylon. Eradication projects were started in India in 1946. By using DDT residual sprays in houses and cattle sheds throughout the Kanara and Dharwar districts of Bombay the spleen index was reduced from 70 per cent to 7 per cent in three years. The plan is to extend the campaign over the entire malarious areas of the Province. In northern India, Terai, once so choked with malaria that half the villages were abandoned, is now getting this disease under control with residual sprays. Ceylon started to work on malaria control in 1945. After a field trial of DDT residual spraying the entire island was included in the 1946 campaign. According to the Ceylon minister of health almost two-thirds of the land was uncultivated due to malaria. One epidemic alone caused 80,000 deaths. The only vector of importance is *Anopheles culcifacies* Giles. DDT treatments repeated about every two months have brought malaria under complete control at a cost per capita of 22 cents a year. Large areas of land have now become available for cultivation and the people are better able to work them.

Thailand's success in largely eliminating malaria over part of the province of Chiengmai was made into a film entitled "The Ancient Curse." Before the eradication program was begun an average of about 2,200 people in the Serapee district were sick with the fever. The vector was found to be *Anopheles mimimus* Theo. Spraying operations were started in all dwellings and other buildings by the Thailand government with ECA financial and technical assistance. As a result, malaria transmission was largely stopped and prosperity increased in this rice-growing area. The WHO-UNICEF project in Thailand protected 40,000 persons in 1950 and the continued efforts of various groups gave malaria protection to 800,000 people in 1951. Plans are laid for a large expansion of the work this year and next year (World Health Organization (17)).

From Indonesia comes the report by D. R. Johnson and J. B. Wilson (8) that ten thousand acres of rich rice land in Java, deserted since the war because of endemic malaria, are being brought back into production after DDT spraying was begun by the Indonesian Ministry of Health and the Mutual Security Agency. An increased production of 4,400 tons of husked rice annually is anticipated from this operation.

Forward steps have been taken in Pakistan. Demonstration teams have applied residual sprays in houses occupied by a quarter of a million people, and the results have been convincing. It is reported that rice yields have increased 15 per cent in areas where well organized malaria control has been carried out.

In Afganistan and Iran much interest is being manifested in the control of *Anopheles* mosquitoes. This has been stimulated by the excellent results shown by residual spray operations made in selected malarious areas. In Afganistan a marked decline in the incidence of malaria has resulted in 1951 from residual spraying in demonstration areas.

Madagascar started a house-spraying program in 1949. About 1 million people have been given protection from malaria. DDT, BHC, and chlordane were combined in an emulsion used in some of the operations.

Malaria mosquitoes came to the island of Mauritius in 1860. Since that time it has been one of the worst malarious spots in the world. A vigorous campaign undertaken in 1945–46 under the direction of M. A. C. Dowling (3) and continued to date, has resulted in a spectacular reduction of malaria. For instance, hospital admissions dropped from 5,014 in 1942 to 196 in 1950. *Anopheles funestus* Giles, one of the chief vectors of malaria, and *Aedes aegypti* (L.) were practically eliminated. Since some *Anopheles gambiae* Giles still persisted, larviciding operations were begun late in 1950 as a supplementary measure.

In South Africa malaria carried by *Anopheles gambiae* and *A. funestus* became so severe that farmers began selling their lands and moving away. Seventy per cent of the labor suffered from malaria during the most critical harvest periods, and crops were lost. In 1945–46 house sprayings with DDT were begun, with the result that 42,000 square miles have been cleared of malaria. Bank and post office business in former malarious areas has been greatly accelerated. Most school absenteeism has disappeared. Farmers have extended their fields and production has been quadrupled, Williams and Stowman (16).

The serious consideration given to malaria problems in Africa and the plans laid for future antimalarial campaigns at the Malaria Conference (18) held in Kampala, Uganda, in November 1950, must be regarded as an important forward movement.

In Egypt DDT residual spraying was begun soon after World War II. In the successful campaign to eliminate an invasion of the Nile Valley by *Anopheles gambiae,* such spraying played an important role.

In our own country malaria has been on the decline for many years, Andrews (1), Russell (13). Since DDT has been used this decline has been accelerated. Today cases of malaria originating in the United States are rare. Probably the greatest factor in cutting down malaria during the last few years was the extensive residual-spray program carried out by the U. S. Public Health Service in cooperation with State health authorities.

We must not overlook the fact that practically all this rapid advance and the optimistic outlook for the future can be credited to mosquito control by the use of the new insecticides, particularly DDT, of which this country alone produced about 78 million pounds in 1950.

Although malaria-control operations are most needed and the results most striking, the value of controlling other mosquitoes should not be underestimated. The development of a protective vaccine against yellow fever has removed some of the urge for yellow-fever mosquito control; nevertheless the advances in mosquito control in South American countries a few years ago have been well maintained. The progress made in several of those countries has surpassed that observed in the United States. The recent outbreaks of jungle yellow fever in Central and South American countries, with little or no trouble in urban areas, indicate that *Aedes aegypti* (L.) control has been a big factor in preventing serious urban outbreaks. They also point to the need for more intensive efforts against this mosquito in Mexico and this country.

Progress in mosquito control in the United States has been notable. Not only has this advance been of a practical nature, but improvement is manifest in techniques, materials, equipment, and organization.

The appearance of strains of mosquitoes resistant to insecticides has caused everyone concerned to give greater attention to practices designed to eliminate mosquito breeding places. Furthermore it has stimulated basic research to solve the resistance problem. This problem is also of great importance in the fight against agricul-

tural and forest pests. This basic work will require time, but it is expected to yield results of great value.

Water control under conditions of heavy rainfall, irrigation, on salt marshes, and in connection with impoundments is receiving renewed interest. The value of water management in mosquito repression is well exemplified in the Tennessee Valley. Early impoundments in northern Alabama produced multitudes of malaria mosquitoes and much malaria. In 1934 blood surveys of people near the Wheeler Reservoir and Lake Wilson showed 25 to 65 per cent of the individuals positive for malaria. Lake-margin clearing, water-levels management, and shore-line maintenance were combined with limited use of larvicides and residual sprays. The tremendous chain of artificial lakes covering more than 600,000 acres with a 10,000-mile shore line, together with provision for flood control, navigation, and power, presents a serious mosquito-disease problem. Even under these conditions malaria is now entirely absent from the Tennessee Valley. Surveys made during the last two years showed not a single positive blood film. F. E. Gartrell (5) of the Tennessee Valley Authority has stated that 8,595 inspections of premises were made for *Anopheles quadrimaculatus* Say during 1950, and the average count was 1.7 females per inspection. This indicates the effectiveness of the antimosquito program.

The extensive soil and water conservation program throughout the country might well precipitate serious malaria problems. However, information acquired by the Tennessee Valley Authority and other agencies should enable us to meet these problems.

In the last few years we have witnessed great advances in insecticide development and improvement in application techniques. These have increased the effectiveness of the treatments, reduced the cost, and lessened the hazards to operators and to the public. The development in equipment for applying aerosols has been particularly outstanding.

The search for new insecticides for mosquito control is being continued by the Bureau of Entomology and Plant Quarantine, the Public Health Service, and several State agencies. Among the new materials found to be effective against mosquito larvae, the organic phosphorus compounds are the most promising. EPN and malathon are among the promising phosphorus compounds that will be explored further in mosquito control. Although most of these materials are highly toxic to animals and man, it is hoped that methods for safe use can be found, and that mosquitoes will not develop resistance to them so rapidly as to the chlorinated hydrocarbons.

In the Alaskan research it was shown that the application of 0.3 to 0.7 pound of DDT per acre to the breeding areas gave a high degree of larval control for two seasons. The DDT residue remaining in a 100-square-mile treated area reduced larval abundance a year later by at least 95 per cent. The Public Health Service has demonstrated a remarkably long-lasting residual effect from dieldrin applied as a prehatch treatment.

Several thousand compounds have been tested as synergists to increase the efficacy of DDT against resistant-mosquito larvae. However, none of them increased the effectiveness of DDT appreciably.

Research on repellents conducted by industry and State and Federal agencies has made distinct progress. The effectiveness and practicability of clothing treatments to supplement skin treatments with standard insect repellents, were investigated in Alaska by the Bureau of Entomology and Plant Quarantine and the Department of Defense. A material, designated M-1960, was found to prevent mosquito biting through clothing for about a week when uniforms were dipped in 5 per cent emulsions of this material. The formula is one developed at Orlando as a multipurpose clothing repellent to protect military personnel from attack by mosquitoes, ticks, mites, and fleas. The concentrate consists of 30 parts benzyl benzoate, 30 parts *N* butyl acetanilide, 3- parts 2-butyl-2-ethyl-1, 3-propanediol and 10 parts emulsifier.

Industrial and Engineering Chemistry (7) reported that D. E. Piper, R. H. Hall and G. F. Wright, University of Toronto, had encouraging results in preliminary tests with a number of compounds as repellents. Among these were *d,l*-benzyl lactate; 70% 2-methyl-1-phenyl-1, 2-propanediol + 30% ethyl mandelate; 1-hydroxy-1-phenyl-2-propanone; 1-methyl-1-phenyl-1, 2-propanediol; ethyl mandelate.

The organization of new mosquito control districts is always encouraging, as it shows that interest in nuisance abatement is spreading. Two new districts have been created in Virginia in 1951. In Utah a South Salt Lake County Mosquito Control District has been set up and another district to include Davis County is being organized. No doubt there are a number of other recently established abatement districts of which I do not have knowledge.

The important bearing that studies of mosquito biology, distribution, and taxonomy have on control work is not always fully recognized. In the study of the life history and habits of a mosquito a means of rearing it in the laboratory must be developed. Much has been done in this direction, as indicated by the excellent summary of mosquito-culture techniques by Helen Louise Trembley (14), to be presented at this meeting. She reports that 43 species have now been successfully colonized, 17 of them since 1944.

It is surprising how many new species are continually being turned up. In 1951, for instance, 47 new species, 2 subspecies and 1 genus, were described. These included 11 *Aedes,* 2 *Anopheles,* and 20 *Culex.* Advances have also been made in determining whether closely related forms, such as those in the *Culex pipens* L. complex, are really distinct species, subspecies, or simply strains.

Information of value on distribution is being accumulated in many areas. Perhaps the most extensive study along this line is that being carried on by C. R. Twinn (15) and associates in the Arctic regions of Canada.

Knowledge of flight habits is of great importance in mosquito control. Notable advances in this direction, especially through the use of radioactive isotopes, have been reported in California with *Culex nigromaculus* (Ludl.) and in Florida by Mulrennan (10) with *Aedes taeniorhynchus* (Wied.). In the Florida tests with *A. taeniorhynchus* a dispersion over a 20-mile area occurred within 3 days after liberation.

Contributions to knowledge of the biology, habits, and ecology of numerous species have been made by many workers. Undoubtedly, investigations such as are now being carried on with *Aedes taeniorhynchus* by Mulrennan (10) and associates in Florida will be of material aid in indicating ways of strengthening our defense against these pests.

A review of the mosquito control situation from a world viewpoint clearly shows a forward movement. The application of known methods of mosquito control to malaria problems is now freeing millions of people from this, the world's most debilitating disease. It is also greatly lessening the hazards of those terrifying diseases, yellow fever, encephalitis, and filariasis. Relief from annoyance and economic losses by pest mosquitoes is also paying substantial dividends. However, the job is far from finished. Years of effort will be required to relieve many parts of the world from the distressing effects of mosquito borne diseases. The efficiency, speed, and perhaps the ultimate success of this effort will depend on a continued flow of new information from basic and applied research. Much needed information is forthcoming, the difficulties encountered are being overcome, and the outlook for the future is good.

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THE PLUMBER'S NIGHTMARE, KING SIZE

THEODORE G. RALEY
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During the last few years insecticidal fogs have been used extensively for the control of adult mosquitoes. Interest in this method of control has stimulated the initiative of many workers, all seeking the same end result. This search has been directed toward an efficient, low-cost fog generator that could cope with the many variables encountered in field operations, operating personnel and available materials. The fog generator described in this paper is Consolidated Mosquito Abatement District's contribution to that search. The Plumber's Nightmare, King Size, has been field-tested and is presented with pride by a group of experienced foggers.

The Plumber's Nightmare is a product of limited income and unlimited mosquito production (Raley, 1947). More odds and ends from the plumber's scrap pile, war-surplus material and discarded vehicle parts than new parts have been used in developing this fog generator. William Miller, of the Sutter-Yuba Mosquito Abatement District, of California, deserves full credit for his determination and his ability to find necessary parts at little or no cost. The mosquitoes were a constant irritating reminder that needled us into many hours of scorched-finger labor.

As the Plumber's Nightmare gained favor as a vehicle exhaust generator, modifications were assembled in many parts of the world. Ralph Crowe expanded its use by building a portable unit for fogging the inside of buildings on Guam (Crowe, 1948). A. L. Fleming, at Sutter-Yuba, met the challenge by modifying the original (Fleming, 1950). This unit is perhaps the most efficient of any fog generator for use on a vehicle. Fleming's modification eliminated practically all "drag" on the truck motor and nearly doubled the rate of material discharge. These improvements made it possible to operate the vehicle in a higher gear, saving both time

and equipment. Other modifications have been installed on several types of trucks. One interesting model is the dual arrangement mounted on a 1½ ton truck by the Bureau of Vector Control. The two "Nightmares" are fixed on the front bumper, pointed in opposite directions. Even passenger cars have been used as a medium for generating the necessary hot air.

Through the years, generators that produce larger volumes of fog than were being produced by vehicles have been used successfully. To my knowledge, Robert H. Peters, Manager of the Northern San Joaquin County M.A.D. of California, unveiled the first fog machine with a Plumber's Nightmare venturi (Peters, 1950). Several other agencies had modified or built fog machines, but the Peters model started a trend that is still resulting in different designs. These early machines had only one fog head and have given good results in all districts that have them.

The two-barreled King Size machine (Fig. 1) is the end-product of several designs built by Consolidated M.A.D. One-barrel units were operating so successfully that it was suggested that two might give better results. Donald Merritt, Consolidated's builder extraordinary, somehow found time in mid-mosquito season to give substance to thought and came up with the control man's delight. This machine has met several tests with flying colors. Experience gained in building the one-barrel machines gave a good background for the basic design. The new modification turned out to be a simple change-over with no loss in mechanical efficiency. Adding the second orifice approximately doubled the output of previous machines built by the district. Only gross observations have been made on the killing qualities of fog produced by the King Size unit. Experience over the years with other Nightmares has shown them to be dependable under conditions suitable for fogging, so we have confidence in the new one. More precise testing is planned during the next mosquito season.

Since the unit is far from an engineer's idea of a precision-built machine, no effort will be made to give more than a general description of the machine. Drawings and pictures have been reproduced and are available on request. (See also Figs. 1 and 2.) In principle, the mechanical features are similar to several other machines. A gasoline engine with a conveniently located fuel supply drives a vane-type blower. The air blast created by the blower is carried to a two-compartment chamber, circulated through the two sections, heated by a gasoline burner, and discharged through the two venturis; insecticides are introduced near the first constriction of the Nightmare. These venturis are oversized (2" x 1¼") with an adjustable insecticide feed line for each. Pumps, gauges, pressure regulator, connecting lines, etc. are added to make the complete unit. Only the one King Size model has been completed. All machines have been remodeled war-surplus York-Hession smoke generators. Except for the frame and gasoline tank every part of the York-Hession has been replaced in one or another machine. This has demonstrated a practical solution for future construction. A new King Size fogger has been started and will be made from readily available standard parts. Full details will be recorded.

The principal features of the King Size Nightmare are favorable, but one bad feature is the lack of positive control of particle size. Adjustable feed lines give a certain degree of control but as yet it is not recommended for use in homes. There have been many hours of operation in and around populated centers with little complaint of automobile spotting or similar damage. When proper adjustments of heat and flow rate are made, the discharge is reasonably constant. Good features are low cost, ease of repair, light weight, quiet operation, low heat range, safety; and volume and quality of fog.

Fogging is, of course, only one phase of a well rounded mosquito control program. Circumstances dictate the part it plays in each particular agency, with the money available usually guiding the deci-

Figs. 1 and 2. Plumber's Nightmare, King Size

sion. Regardless of the part it plays, cost, particularly the initial cost, is probably the prime concern of everyone involved. Oftentimes, too few machines will create a greater problem than none at all, so initial investment is of prime concern. Maintenance and operation costs then enter the picture and raise the question of repairs, availability of parts, personnel qualified both to make repairs and operate the machine. These factors govern the extent to which the machine can be used.

Practical experience in Consolidated M.A.D. indicates that the Plumber's Nightmare King Size is comparatively low in cost. From the experience gained in building eight fog generators that required making or purchasing every vital part, we consider that a new unit can be made ready for field use for less than $500.00. Materials will cost from $375.00 to $400.00 and a qualified mechanic can put them together in 3 to 5 days. Ready accessibility to all parts plus simple construction means low maintenance expense. A good operator can learn to take it apart and make necessary repairs very quickly. As the unit is light in weight (approximately 200 pounds) it can be maneuvered into just about any type of situation, increasing the working time many hours beyond the heavier machine that is restricted to improved roads.

Safety was an important consideration in our approach to the problem of design. By circulating forced air from the blower through the outer chamber of the burner pot, outside temperatures are kept very low. Even after an hour of running time there is little danger of being burned on contact with the pot. The only extreme heat is at the bend in the discharge pipe. Releasing all the air produced by the blower prevents any build up of pressure within the heat chamber, so there seems little chance for an explosion. These features combine to make the machine's operation relatively quiet. The noise from the gasoline motor and the pulleys covers all other sound. It sounds like a sewing machine. The position of the gasoline burner and the free flow of hot air and location of insecticide delivery both reduce the fire hazard.

The low heat and constant release of all air moved by the blower produces a good volume of fog. A few tests have indicated a fairly constant particle size with a very small percentage below one micron. Some smoke is produced, but the greatest amount of the material discharged is over ten microns. Breakdown is complete enough to keep the large droplets to a minimum. The ground directly below the orifice shows no sign of wetting unless the machine is in one spot for several minutes. The airborne fog is wet, and comes close to being a fine mist. The low temperature should make it possible to use a greater variety of insecticides than fog generators with higher temperatures. Pyrethrins have been used only casually as an addition to the standard fog mixture. In the coming season pyrethrum formulations alone will be tested to see how effective they are.

Summarizing, the machine described and illustrated has operated successfully in the field. The original Plumber's Nightmare has been proved by time; however, the King Size is still too new to make precise recommendations. Others who might become involved in building a unit will find the information useful as a guide to a good machine, but we sincerely hope that they will come up with many improvements.

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SOME TECHNIQUES USED IN THE STUDY OF *AEDES* EGGS IN IRRIGATED PASTURES IN CALIFORNIA

RICHARD C. HUSBANDS [1]

Ecological investigations into the problems pertaining to mosquitoes produced by irrigated pastures have been in progress in California for several seasons. As these investigations developed it became apparent that more information was needed pertaining to the more complex aspects of mosquito ecology, and especially about that often neglected portion of the life history of mosquitoes, the egg stage. For this reason, special emphasis was placed upon the study of *Aedes* eggs during the 1951 season. In order to accomplish this work it was necessary to develop improved techniques for the rapid and accurate measurement of egg distribution and densities. Previous experiences with this problem have indicated several experimental approaches to the subject.

Many workers in the field of mosquito ecology have developed improved methods of sampling areas for the presence of mosquito eggs. Gjullin (1938) gave a method of egg separation that used a mechanical seed cleaner with special screens installed for that purpose. Gjullin, *et al.* (1941), Filsinger (1940), Connell (1941), Abdel-Malek (1948), Horsfall (1949), and Bodman and Gannon (1950), devised methods of investigating egg distribution or showed the relationship of distribution to factors which influence egg hatch, such as temperature, dissolved oxygen, and plant hormones. Although this work was basic to approach the study of *Aedes* eggs associated with irrigated pastures, none of the techniques proved to be completely applicable to the conditions and species encountered in pastures.

Aedes nigromaculis (Ludlow) constitutes the major species present in irrigated pastures in California and produces a brood of adults with each irrigation. Eggs are produced within three to four days after the mass emergence of a brood. After these eggs mature, hatching can occur within fifteen minutes after water is applied to the field, and if the eggs are not conditioned by environmental factors to prevent hatching, a majority of the eggs will hatch with each irrigation. A measurement of relative egg densities can be obtained by flooding samples of the pasture, but several unknowns, such as the influence of temperature or seasonal variations, make this procedure questionable. Actual densities can therefore be measured best by actual counts of eggs present, and this can only be accomplished by the removal of a portion of the soil surface for screening and examination.

Methods used by previous workers required that a sample of soil be removed from the area of study by cutting to a predetermined depth and removing the sod for flooding or for drying and screening. The best samples that could be obtained by this method were generally from one to two inches thick, and since the surface of a pasture is relatively stable and because eggs could not be deposited below this surface to a very great depth, a thick sample would contain much soil that would mask the eggs present because of the dilution factor of the soil. Therefore, in order to overcome this problem which produced an excess of soil and destroyed the plants in the area sampled, a method was devised which used a tank type vacuum cleaner to remove the sample surface. A portable generator mounted in a jeep made the unit mobile so that samples could be taken from the field under most conditions. The cleaner was modified by substituting a hose with a smooth lining for the regular extension tube, and samples were collected within the cleaner in small cloth bags which prevented abrasion and damaging agitation of the eggs. Tests

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FIGURE 1. Rotary Stage used in the examination of soil samples for the presence of *Aedes* eggs. Photo T. D. Mulhern

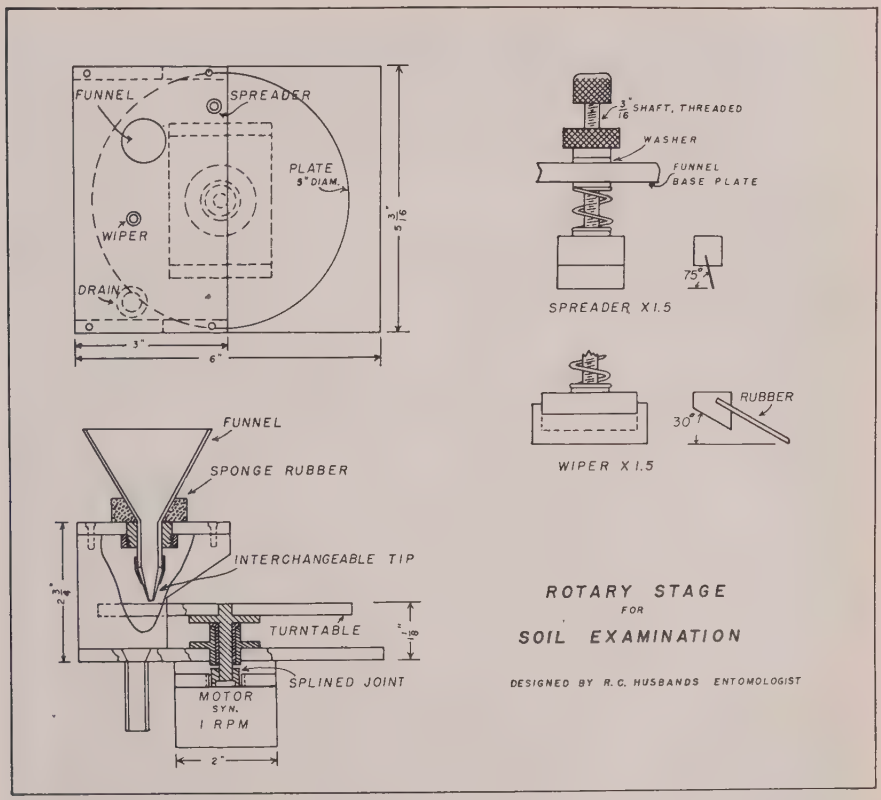

FIGURE 2. Diagram for the construction of the Rotary Microscope Stage for soil examination.

showed that less than 0.1 per cent of the eggs collected by this method were destroyed by vacuum collection. In order to take a sample the covering grass was clipped short over the area to be sampled, and the soil surface was raked loose to a shallow depth before vacuuming. The samples taken by this method could be limited to very narrow layers without disturbing the root growth of the plants within the area of the sample, and the volume of soil taken by this method seldom exceeded a pint of soil per square foot of surface examined. After drying the sample to a consistency suitable for screening (not excessive drying) the sample was easily hand-screened because the soil particles were separated during vacuuming which allowed the eggs and soil to pass freely through the screens. *Aedes nigromaculis* and *Aedes dorsalis* (Meigen) eggs will pass through a 60-mesh screen and will be retained by an 80-mesh screen.

The examination of screened soil samples by the usual method of pouring thin streams of soil upon a white card for examination under the microscope proved to be slow and inaccurate. In order to overcome this difficulty a rotary microscope stage was developed which mechanically applied a thin layer of soil to a white disk which in turn moved the sample into the field of vision in a continuous stream (Figure 1). This stream of moving particles can be examined carefully and can be stopped when needed to remove eggs or

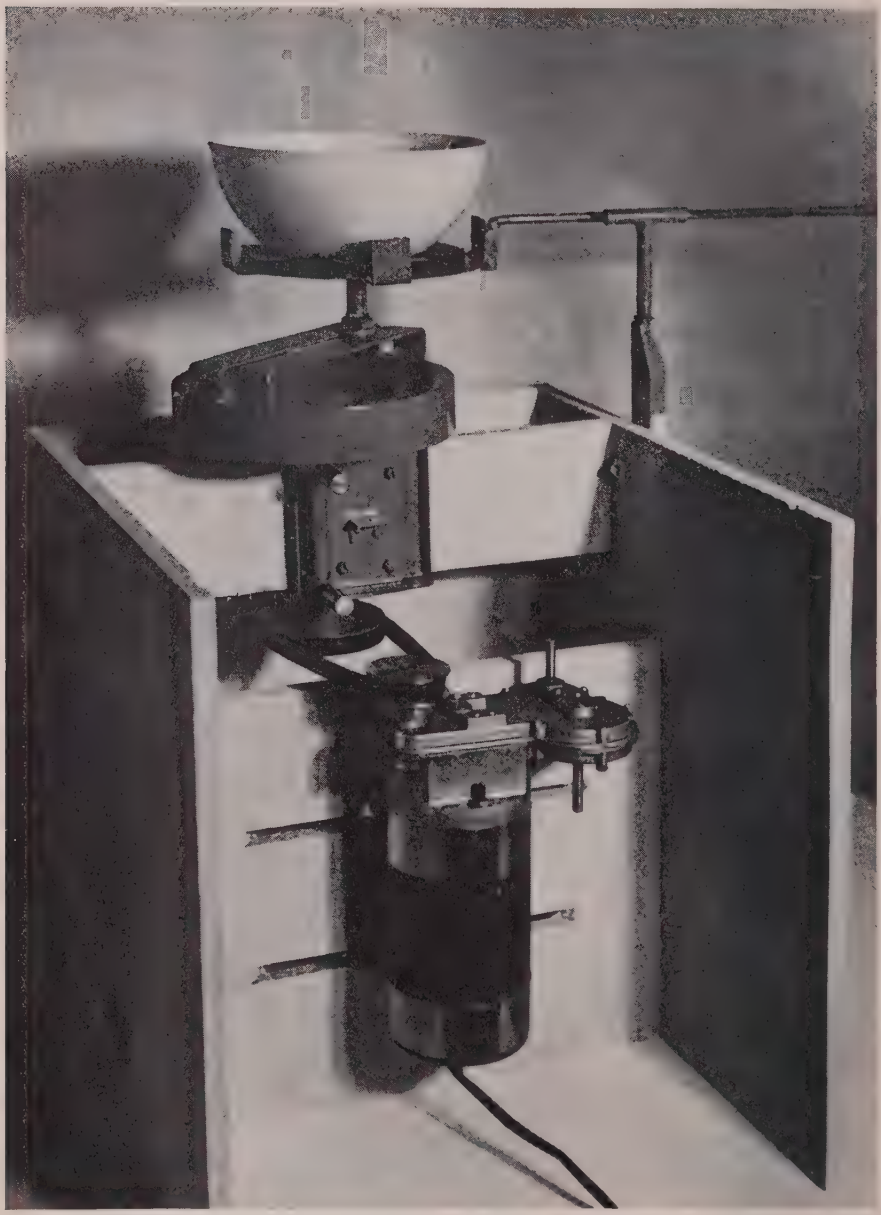

FIGURE 3. Flotation Separator used in the recovery of *Aedes* eggs from laying media when the eggs are laid below the surface.

Photo T. D. Mulhern

for more careful examination of questionable material. The re-examination of soil which was handled by the non-mechanical method showed that eggs were overlooked in the original process, and such samples generally contained eggs when given the more accurate technique of examination. Two-thirds of the samples examined by the rotary stage method contained whole or hatched eggs. The construction of a rotary stage for this type of work is simple and is illustrated in figure 2.

During the process of attempting to develop better techniques for recovering eggs from soil samples a flotation process was investigated. This process proved to be successful but time-consuming when applied to soil containing finely divided organic material or clay particles. When applied to the recovery of newly laid eggs which were deposited in white sand the flotation process proved highly successful. Caged *Aedes nigromaculis* females will deposit their eggs in moist white sand and most of the eggs are visible although they generally may be deposited just below the surface. In making counts of eggs laid in sand it was found that in many cases a small percentage of the eggs were deposited below the surface to a depth that made them invisible from above. By placing the laying media in the flotation separator (Figure 3), such eggs were quickly brought to the surface and accurate counts were obtained. The accuracy of this process could be demonstrated by placing a single egg below the surface of clean sand in the flotation separator. One minute after the rotation process was started the egg was visible on the surface of the sand.

The flotation separator for the recovery of mosquito eggs from soil is based upon the principle that the continuous motion of soil particles in the presence of a liquid medium (water) and at a speed which will produce a stratification of the particles will cause a segregation of the particles dependent upon their specific gravity and shape. At the proper speed of action the light particles will work to the top or surface. Mosquito eggs will be found on the upper surface under such conditions, since they are much lighter than most soil particles.

In order to obtain this separation a special rotary mechanism was designed [1] which moved a quart-sized bowl in a rotary path at about 60 rpm. The motion of the bowl can best be described as similar to that used in panning for gold. A mechanical separator is not necessary since the same motion can be obtained by holding the bowl in one hand and rotating the bowl at approximately the proper speed. The soil or sand in the bowl is covered with about one inch of water. Hatching can be prevented by holding the sample of soil at 33° F. for ten days and by using very cold water to cover the eggs.

As mentioned before, separation in clean sand is more satisfactory than in soil. When soil is used the eggs will be hidden under a layer of slower settling residue. This layer with the eggs can be removed with a pipette and filtered to recover the eggs. This process is time consuming and not accurate, and therefore it is recommended that, except in special cases, the examination of soil for eggs be carried out by means of the rotary stage technique.

Summary and Conclusions

1) A portable tank type of vacuum cleaner can be used successfully for collecting soil samples for the study of *Aedes* eggs.

2) A great increase in the accuracy of examining screened soil samples for *Aedes* eggs can be obtained by the use of a mechanically rotated microscope stage to which a very thin layer of the soil is continuously applied.

3) A flotation separator can be used to facilitate the accurate counting of eggs laid in artificial media such as clean white sand.

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THE BUREAU OF ENTOMOLOGY AND PLANT QUARANTINE'S CONTRIBUTION TO RESEARCH ON MOSQUITOES DURING 1951

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The research on mosquitoes carried on by the Bureau of Entomology and Plant Quarantine during 1951 is briefly summarized under the headings "Taxonomy" and "Biology and Control" as in my report for last year. Again this information has been made available to me by Alan Stone, taxonomist in Washington, by A. W. Lindquist, in charge of our Corvallis, Oregon, laboratory, and by W. C. McDuffie, in charge of our Orlando, Florida, laboratory. W. V. King, who was formerly in charge at Orlando, has been assigned new duties. They consist of analyzing data, revising and bringing up to date old publications, and preparing new manuscripts giving data on the results of research conducted at that station. As reported last year, the taxonomic investigations and those conducted at Corvallis are financed by regular appropriations from Congress, whereas those at Orlando are financed by funds allotted by the Department of Defense.

TAXONOMY

Research at the United States National Museum. Alan Stone described a new species of *Aedes* from Tahiti early in 1952. Kathryn Sommerman was engaged to prepare pictorial keys to mosquitoes of medical importance in several overseas areas for use by the Army. Her work is financed by funds allotted the Bureau by the Department of the Army. A number of outside workers have been aided in their taxonomic studies, the most important being H. R. Foote in his study of the larvae of *Culex* (*Melanoconion*) species, E. F. Cooke in his study of the *Chaoborinae,* Marion Smith in her study of a new species of *Aedes* (*pseudodiantaeus*), G. W. Lassman in his description of a new *Psorophora,* and Elizabeth L. Marks in her study of *Aedes scutellaris* and its relatives. Several entomologists studied the mosquito collections at the United States National Museum before going to overseas assignments in the Orient and in Africa.

BIOLOGY AND CONTROL

Research at the Corvallis, Oregon, Laboratory. *Mosquito resistance to insecticides.* —As far as can be determined, insecticides are satisfactorily controlling *Aedes* mos-

quitoes in the Pacific Northwest. As yet there are no indications that the floodwater species *A. vexans* and *A. sticticus* are developing resistance to DDT, but it is entirely possible they may do so in the future. Comparatively little control has been attempted against *A. dorsalis* in the irrigated sections of Washington and Oregon, hence it is much too soon to observe resistance by this species in that region.

In California, however, the situation is much different. *Aedes nigromaculis, A. dorsalis,* and *Culex tarsalis* are definitely resistant to DDT over a wide area in the San Joaquin Valley. Considerable specific information on this subject was obtained by a cooperative study with the California Department of Public Health's Bureau of Vector Control. C. M. Gjullin, of our Bureau, spent several months on this problem with the help of personnel assigned by the Bureau of Vector Control. Carefully designed experiments demonstrated that larvae of the species mentioned from treated fields had built up as much as 12 times more resistance to DDT than comparable larvae from untreated fields. Some resistance by *Aedes nigromaculis* to toxaphene, dieldrin, and aldrin was also observed. As the details of this research are to be reported by Gjullin and Peters, they will not be given here.

During the past two summers research has been directed towards controlling adult *Aedes* in the vicinity of summer homes around Forest Service installations and resort areas in the Cascade Mountains. Such areas have been protected from mosquito annoyance during daylight hours by applying about 2 pounds of DDT per acre over 1- to 5-acre tracts. A single treatment remained effective for several weeks. During a 2-hour period just before and after dusk, when the mosquitoes migrate from one place to another, the DDT residual treatment did not provide satisfactory control. Portable gasoline-powered sprayers were found to be satisfactory for applying this residual spray in bushy overgrowth. Pyrethrum and allethrin were found to be equally effective for the temporary control of mosquitoes during the dusk migration period. A. W. Lindquist has reported on the details of this research.

New chemicals for use as mosquito larvicides were tested in the laboratory, and efforts were made toward improving some of the old ones. None of the new chemicals were outstanding. Three new synergists for DDT improved the efficacy of the chlorinated hydrocarbon insecticides when tested against *Aedes* mosquito larvae resistant to DDT. However, they did not completely nullify the resistance.

A. R. Roth has operated a New Jersey light trap at Summer Lake, Oregon, during June and July 1950 and July and August 1951. His data on collections from an area where little information has been reported on mosquito ecology are rather interesting (Table 1).

Table 1.—Average number of mosquitoes caught nightly, Summer Lake, Oregon.

Species [1]	1950		1951	
	June	July	July	August
Aedes dorsalis	147	283	69	53
Culex tarsalis	32	382	141	120
Culiseta inornata	29	73	281	203
Anopheles freeborni	7	44	10	13

[1] A few *Aedes campestris* and *A. flavescens* were also taken.

Research at the Orlando, Florida, Laboratory. Of the thousands of chemicals that have been tested as mosquito repellents, less than a score have proved highly effective and safe for unrestricted application to the skin. Our experience has shown that mixtures of several good repellents are effective against a wider range of species and on more individuals than any one repellent used alone.

Some of the more effective mixtures against several species of *Aedes* are (1) dimethyl phthalate 4 parts, 2-ethyl-1, 3-hexanediol 3 parts, and dimethyl carbate 3 parts; (2) dimethyl phthalate 4 parts, 2-ethyl-1, 3-hexanediol 3 parts, and *N,N*-diethylsuccinamate 3 parts.

Several of the most effective mixtures against *Aedes* mosquitoes proved relatively ineffective against *Anopheles quadrimaculatus.*

A number of laboratory and field tests were made with insecticidal materials and formulations in areas where salt-marsh mosquitoes were moderately or highly resistant to DDT. Emulsions of dieldrin were slightly more effective than emulsions of chlordane, and both were considerably more effective than pellets containing the same materials. Nearly perfect control of *Aedes sollicitans* and *A. taeniorhynchus,* both highly resistant to DDT, was obtained with ground sprayers applying from 0.05 to 0.2 pound of dieldrin per acre. Airplane sprays were ineffective where dieldrin was applied at the rate of 0.05 and 0.1 pound per acre, whereas at a dosage of 0.2 pound they gave nearly perfect kills. The poor results obtained with the lower dosages were no doubt due to poor penetration of the insecticide through the foliage.

Additional field tests were made against salt-marsh mosquito larvae resistant to DDT to determine the effectiveness of several chemicals that had shown promise as synergists of DDT. Emulsions containing 5 parts of DDT and 1 part of synergist were applied at the rate of 5 pints per acre. Monoethyl phosphate was the only chemical that increased the effectiveness of DDT, and this increase was too small to be of practical significance. Some of the synergists tested were isobutyl acetate, phenyl diphenylphosphonate, 1-(3,4-dichlorobenzoyl)-2 phenylhydrazine, and DMC.

An interesting experiment was made in which the susceptibility of *Aedes taeniorhynchus* larvae from heavily treated marshes of Brevard and Sarasota Counties was compared with that of larvae from the occasionally treated Titusville Beach marsh in Brevard County. Larvae from each of the two areas were treated with DDT, lindane, and dieldrin. The larvae from the heavily treated marshes showed a higher degree of resistance to DDT and were slightly more resistant to lindane and dieldrin than were the larvae from the lightly treated breeding areas.

A series of tests was made to determine the effectiveness and practicability of outdoor residual treatments of several insecticides against *Aedes taeniorhynchus* and *A. sollicitans* adults around isolated 2-acre home plots. Lindane and BHC containing 40 per cent of the gamma isomer, the most effective materials tested, gave good control for 17 days when applied at a dosage as low as 0.5 pound of the chemical per acre. Dieldrin was comparable in effectiveness for 13 days when applied at the rate of 2 pounds per acre. DDT and chlordane were the least effective of the insecticides tested, although at 1 and 2 pounds per acre both gave good controls for 3 to 7 days after treatment. These treatments greatly reduced daytime annoyance from mosquitoes, but during the evening when mosquitoes were active they were observed biting and the treatments appeared ineffective. It was apparent, however, that the mosquitoes were destroyed after resting several hours among the treated foliage in the vicinity of the home plot. When the pressure of the mosquito population outside the treated plot was sufficiently great, the mosquitoes would move into the treated area but did not die until after they had severely annoyed the occupants of the area.

Considerable data were obtained on the effectiveness of prehatching treatments in areas where the salt-marsh larvae were resistant to DDT. Dieldrin emulsions or wettable-powder suspensions applied at the rates of 0.5 and 1.0 pound of dieldrin per acre gave satisfactory control after three and four floodings. The suspensions also gave good control after two floodings when applied at the rate of 0.2 pound of dieldrin per acre. Emulsions at 0.1 and 0.2 pound of dieldrin and suspensions at 0.1 pound of dieldrin per acre were relatively ineffective.

Toxaphene emulsions and suspensions at 1.0 pound of toxaphene per acre gave excellent control after three floodings. The emulsions gave inconsistent results at lower dosages, but excellent control was obtained with the suspensions at 0.2 pound per acre after one flooding.

SOME ASPECTS OF MOSQUITO CONTROL ACTIVITIES IN SOUTH AMERICA

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In this brief comment on mosquito control work in South America I shall only be able to touch upon activities in a few countries. There are eight independent republics and five dependencies of European powers in the area, ranging in size from tiny Tobago with 116 sq. miles to huge Brazil which is larger than the continental United States. Conditions vary widely over this vast territory, but all countries except Uruguay lie wholly or partly within the tropics, and all are confronted with the necessity for combating one or more arthropod-borne diseases, of which malaria is the most widespread. Hence nearly all countries which have organized mosquito control campaigns have done so primarily with the idea of malaria control. These campaigns have expanded rapidly within the past six years. The expansion and extension of control programs have been due principally to the availability and efficacy of the residual insecticides.

Most of the countries have very limited financial resources. The amount of money available for mosquito control is therefore relatively small, so that until recently the control programs were largely limited to the use of larvicides and minor drainage, with a small amount of major drainage if there were any funds left over. These campaigns were conducted almost entirely around the more important centers of population, progress was slow, and while results in small areas were good, on the whole the outlook was anything but encouraging.

All this has now been changed. Pessimism has given way to optimism. With the dramatic results being obtained, more money is being appropriated for the programs, and more work is being done. Control programs are being extended to entire countries, and if the present rate of progress is maintained, malaria will soon become a rarity.

The expansion of control programs has been aided by international and by United States governmental agencies. The Pan American Sanitary Bureau, an organization of the American States themselves, is actively supporting a continental *Aedes aegypti* eradication program, and is interested in the control of various arthropod-borne diseases in a number of countries. In addition the World Health Organization, of which the Pan American Sanitary Bureau is the regional office, is directly interested in campaigns in certain countries. An affiliated organization, UNICEF, furnishes residual insecticides to some countries, which would otherwise have difficulty in obtaining them in sufficient quantities. The IIAA, a U. S. Government organization which administers the Point Four program, gives financial aid and expert assistance in various countries. The Rockefeller Foundation, one of whose principal interests used to be in malaria, still gives a small amount of aid to two or three countries.

However, the countries themselves are directing their programs and are doing a good job of it. Over the years they have developed a strong body of administrators and technical men, many of whom are known to members of this Association.

Their task has not been easy. It is perhaps difficult for most of us to visualize their problems, political, geographical, financial and social. In a few instances, politics may dictate the hiring of personnel and the places where campaigns shall be conducted. In most, communications are difficult, roads few or non-existent in the malarious areas, and the centers of population small and often isolated. More serious, perhaps, is the fact that—with a few exceptions—the educational standards

are low, with a high rate of illiteracy, so that not only is it difficult to reach the people by the usual educational methods, but what is worse, it is difficult to find subordinate employees who can accept responsibility and who can understand the importance of the work they are called upon to perform.

The professional classes are well educated, however, and the general principles upon which control work is conducted are the same throughout the hemisphere, thanks to the free exchange of information which we enjoy. This exchange has gone both ways, for each continent has profited by the experience gained in the other.

I shall now take up briefly a few of the accomplishments in mosquito control in three countries, while emphasizing that equally good work is being done elsewhere.

Brazil. Under Doctor Mario Pinotti, Brazil has developed the largest control organization in this hemisphere, covering as it does the entire country with the exception of the State of São Paulo. They work from the vast and difficult Amazonian region in the north to the extensive and difficult forest areas of the State of Santa Catarina in the south.

This latter region is unique. It is the only place in the world outside of a small area in Trinidad, where severe endemic malaria is known to be caused by *Anopheles* breeding in bromeliads. The area covers some 40,000 sq. km., or more than 15,000 sq. miles, in a wide strip along the mountainous coast. Important as an agricultural and industrial region, it is inhabited by almost a million people. Bromeliads are epiphytic plants somewhat similar to and related to the common pineapple. Most species hold water in their leaf bracts, varying in quantity from a teaspoonful to a quart or even more. It is in this water that certain species of mosquitoes breed, among them *Anopheles* of the sub-genus *Kertezia*. The bromeliads may be either terrestrial or arboreal, depending upon the species.

Thus the malaria in the city of Florianopolis, a city of 35,000, was carried by *Kertezias* breeding mainly in ground bromeliads. Hand removal of these plants was begun in 1943 and in 6 years all the 15 million plants had been destroyed over an area of 33 sq. km. Malaria has been wiped out. The cost was close to 7 cents per plant.

This was a fairly simple operation, but the problem in the heavily forested areas just back of the coast is much more difficult. This is mostly virgin rain forest, with the larger trees reaching a height of over 100 feet. The trees bear a heavy population of bromeliads, often with hundreds of plants per tree, or as Pinotti puts it, an average of 5 plants per sq. meter.

There are only two ways to prevent mosquito breeding—either destroy the trees and with them the bromeliads, or destroy the bromeliads only. Neither can be done over the whole area by the Malaria Service, but a good deal is being done to control the malaria. As the region is settled and the resources utilized, the people themselves are cutting down the trees either for lumber, or to clear the land for their farms. The Malaria Service has cut down the forest around the more important cities such as Blumenau, Brusque and Joinville, and the famous baths of Caldas da Emperatriz, and is reforesting with smooth bark trees which are less susceptible to infestation with bromeliads. In other areas, they have carried out the difficult feat of removing the plants by hand, a task which must be seen to be believed. I have seen some of their operators climbing around in the large trees with complete disregard for their safety. Unfortunately, a few serious accidents have happened. These operations have eliminated malaria as a real problem in the principal centers.

The remainder of the population lives in small villages or in isolated rural houses, principally the latter. These houses are regularly sprayed with a residual insecticide, which operation alone has considerably reduced malaria incidence.

The Malaria Service is enlarging its activities to include control of other arthro-

pod-borne diseases, the most important of which is Chagas' disease. This disease is caused by the American trypanosome, *T. cruzi,* and is transmitted by blood-sucking *Triatoma* bugs which live in the house walls, sometimes in incredible numbers. Residual spraying is proving effective. In one campaign in Minas Gerais the Service is spraying some 100,000 houses with parathion.

Naturally, the consumption of insecticides is enormous. Until recently, all supplies were imported, but in 1950 in an effort to become at least partially self-sufficient, the Malaria Institute began the manufacture of gammexane in its own factory. The production goal for 1951 was 600 tons.

Venezuela. The accomplishments of the Malaria Division of the Ministry of Health of Venezuela are well known to most of you. Approximately 90 per cent of the 3 million people living in the malarious zones, which cover some 600,000 sq. km. (over 230,000 sq. mi., or slightly less than the state of Texas) are now protected by regular spraying of their houses with residual insecticides. The area includes the sparsely settled outlying territories, where the job has been really difficult. Trucks, boats, and horses are used for transportation, while a good part of the work must be done on foot. Malaria has been reduced to a very low level throughout the country, and has been wiped out over a large area. The Malaria Division is frankly aiming at malaria eradication.

However, malaria had been eliminated in most cities of over 10,000 population before the advent of DDT, by major drainage, and the Malaria Division continues to spend approximately 25 per cent of its budget on engineering works. One of the reasons, but not the only reason, for continuing permanent measures, is that in the extensive area where *Anopheles albimanus* is the principal vector, the number of *Anopheles* has been reduced little if at all by residual spraying of the houses. On the other hand, the dangerous vector, *A. darlingi,* which is more domestic, is eradicated by residual spraying.

Just as in the case of Brazil, the Malaria Division is being entrusted with additional responsibilities. These include the *Aedes aegypti* eradication campaign, control of rats in urban areas, and fly control as a means of reducing the incidence of gastro-intestinal disturbances. The transfer of other activities to the Division is also being considered.

Tobago. For the past four years a little-publicized campaign for the control and possible eradication of malaria and the responsible species of *Anopheles* has been in progress in the little island of Tobago, with most encouraging results. This work is being done on a very modest budget with funds almost entirely from local revenues.

Tobago lies near and is a ward of Trinidad. It has an area of 116 sq. miles, and a population of 30,000. Most of the island is mountainous, but the western fourth is flat, with several large tidal swamps comprising some hundreds of acres where *Anopheles* breed continuously and abundantly. These and smaller swamps, together with a hundred small streams the mouths of which are silted up by tidal action during the dry season, and thousands of small meadow pools formed during the rains, are the principal breeding places of *A. aquasalis,* the vector species.

With an annual budget of $40,000, or slightly over $1 per person, the Malaria Division of Trinidad and Tobago began its control program in 1948. Each of the 8,000 houses was sprayed with a residual insecticide, on a semi-annual basis. A continuous and intensive anti-larval program was begun, oil being used as a larvicide. These activities continue, but when at the end of the first year *Anopheles* were still being found, it was decided to drain the swamps and open new and protected mouths for the principal streams. By the end of 1951 all except two of the larger swamps had been drained and the rivers were no longer a serious problem.

The results have been dramatic. Malaria has disappeared, land values have

risen enormously, and the island has become a health resort. For almost a year no adult *Anopheles* have been found in the numerous traps or elsewhere, but an occasional larva is still found in the remaining water collections, the swamps and temporary small pools. Dr. Horace Gillette, the Director of the Malaria Division, believes that the species can be eradicated in another two years. Even should he prove too optimistic, the campaign has already more than justified itself.

These are only a few examples of the solid control work being done in South America, and Central America. If time had been available, I should have mentioned also the active and fruitful field and laboratory investigations being carried out in connection with the control activities. However, most of the published reports are abstracted in *Mosquito News* or in other journals.

As one result of the universal use of residual insecticides, the old malaria organizations are being given responsibility for the control of other arthropod-borne diseases, such as yellow fever, filariasis, typhus, plague, trypanosomiasis, bartonellosis, and fly-borne gastro-intestinal diseases. This is a logical step, and one to which I am sure the members of this Association are giving increasing attention.

While the enthusiasm for the residual insecticides is justified in spite of the rapid development of resistance by many species of insects—even including certain species of *Anopheles*—I believe it is significant that the use of "permanent" measures of control has not been abandoned, and in some instances is being increased. I need not go into the arguments for or against the exclusive use of residual insecticides, but I submit that they are to be considered as an additional powerful weapon in the continuing fight against pest insects and insects that transmit diseases to man, and not as the final and complete answer to the complex problem of their control.

FOREST MOSQUITO STUDIES IN AN ENDEMIC YELLOW FEVER AREA IN PANAMA

STANLEY J. CARPENTER,[1] PEDRO GALINDO,[2] and HAROLD TRAPIDO[3]

Although sylvan yellow fever has been prevalent for many years in several countries in northern South America, particularly in adjacent Colombia, its presence in Panama was not generally known until January, 1949. Reports by Clark (1938) and Kumm and Crawford (1943) called attention to the fact that yellow fever had been active in relatively recent years east of the Canal Zone, but it required the actual recognition of several human deaths caused by yellow fever to cause this disease to be recognized as an important problem in Panama.

Entomologists had been active in the Canal Zone and adjacent Republic of Panama during most of the first half of this century, but they were concerned chiefly with the mosquito vectors of malaria. Thus, little was known of the composition of the mosquito fauna and its activity throughout the extensive forests covering much of the country. Following the recognition, in January 1949, of several deaths caused by sylvan yellow fever in the Pacora area, some twenty miles east of Panama City, the writers undertook a study of the composition and activities of the diurnal forest mosquitoes that relate to the possible vectorship by these insects of sylvan yellow fever in this area. At first,

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during the year 1949, the study was limited to areas in the Canal Zone and in Central Panama, but later, during 1950, was extended to western Panama and adjacent Costa Rica, as the disease showed signs of moving westward.

Sylvan yellow fever is principally a disease of monkeys in the deep forests, transmitted from monkey to monkey by mosquitoes living mostly in the forest canopy. The disease is occasionally transmitted to humans, particularly hunters, lumbermen and those engaged in farming small cultivated areas adjacent to the heavy forests.

In nearby Colombia, *Haemagogus spegazzinii falco* Kumm *et al.* and *Aedes leucocelaenus* Dyar and Shannon have been found infected with yellow fever virus in nature and have transmitted the virus by bite. In addition, the virus has been recovered by inoculation from a mixed group of sabethine mosquitoes, including *Sabethes* (*Sabethoides*), *Limatus, Wyeomyia* and *Trichoprosopon* (Shannon *et al.*, 1938). Evidence obtained both from field and laboratory work in South America has incriminated *Haemagogus spegazzinii* and *Aedes leucocelaenus* as the most important vectors of sylvan yellow fever in that area.

A few years ago, Komp (personal communication) had obtained a few larvae of *H. spegazzinii falco* in a tree-hole on Barro Colorado Island in the Canal Zone, but the species was not found again. The species was first recorded in the literature from Panama by Galindo *et al.*, (1949) and described as a common forest species on the heavily forested slopes of Cerro La Victoria where sylvan yellow fever was first recognized in Panama in 1949.

Methods of Study. During January, 1949, a series of four mosquito collecting stations was established in the heavy forests, from about sea-level to 2100 feet altitude, in the Pacora area, near the location where several fatal cases of yellow fever had occurred. In addition, one station was established in a heavy rain forest near Fort Sherman, on the Caribbean side, and another at Juan Mina on the Chagres River, midway across the Isthmus. Each of these stations consisted of a tree in the dense forest to which platforms were attached about midway up the trunk and in the crown of the tree. These platforms were made accessible from the ground by strong ladders. The upper platform at these stations ranged from 40 feet to 71 feet above the ground, depending on the height of the trees in each particular locality. During 1951 and 1952 all stations were equipped with a single platform located in the forest canopy (Figure 1).

Simultaneous catches were made of mosquitoes either biting or approaching to bite human collectors stationed at ground level and on the platforms, one day each week at each station (Figures 2 and 3). During the first year of study, thirty-minute collections were made each hour from 8:00 a.m. to 5:30 p.m. Common species of ground pool breeding mosquitoes, including *Aedes taeniorhynchus* (Wiedemann), *A. serratus* (Theobald), *Mansonia* spp. and *Psorophora* spp., were usually captured in chloroform tubes and then transferred to appropriately labeled pill boxes for subsequent identification. The collectors soon learned to recognize metallic colored species and other true forest mosquitoes, and these were allowed to feed and were then captured in small oviposition vials, each provided with a wad of moist cotton and a circle of filter paper on the bottom. The vials containing the live specimens were carried to the laboratory, where the females were held for oviposition. Much valuable information relating to their identity and habits was obtained from these ovipositing females and their progeny (Galindo *et al.*, 1950).

Information was obtained on the breeding habits of mosquitoes in the forest by making weekly collections of larvae from nearby tree-holes and miscellaneous water containers in each station area, and through the use of bamboo larval traps, hung on the station tree and nearby trees (Galindo *et al.*, 1951a).

Two types of bamboo larval traps were used in these studies, those with the top open (Figure 4), and those with the top covered with a lid or hood, and a small

FIG. 1. Tree station used for collecting mosquitoes in the forest. Ladder, platform in forest canopy, and assistant used as bait and collector are shown.

FIG. 2. Collecting mosquitoes on platform in canopy of the forest.

FIG. 3. Collecting mosquitoes at ground level at tree station in the forest.

hole cut through the side (Figure 5). We realized early during this work that we were not getting larvae of *Haemagogus spegazzinii falco* and *Sabethes* spp. in numbers commensurate with the adult catches of these species, and the closed top bamboo trap, with the small opening through the side, was designed in an attempt to meet the breeding requirements of these mosquitoes. This type of larval trap solved our problem with *Sabethes cyaneus* (Fabricius) and *S. chloropterus* (Humboldt) but apparently did not meet the breeding requirements of *H. spegazzinii falco.*

The bamboo traps were examined for larvae once each week at each station. When larvae were found, they were transferred to appropriate containers and carried to the laboratory for immediate identification or for rearing and associating the adult with the larval and pupal exuviae. The bamboo traps with the tops open were left empty after each weekly examination during the rainy season, to be refilled by rainfall. It was necessary to carry water and refill these bamboo traps by hand during the dry season. Bamboo traps with the closed top had to be refilled by hand each week during both the dry and rainy seasons.

Several additional adult mosquito collecting stations were established and operated for specific purposes during brief periods of the first year of the study. Four such tree stations, consisting of a single platform each, were established near Buena Vista, between the Trans-Isthmian Highway and Gatun Lake, where two fatal cases of yellow fever occurred in August, 1949. Unsuccessful attempts were made to recover yellow fever virus from mosquitoes captured at these tree stations during the remaining few weeks of the rainy season. Six similar stations were established and operated simultaneously for this purpose on Cerro La Victoria near Pacora.

One station was established in a mangrove swamp near the ruins of Old Panama for the purpose of studying the habits of *Haemagogus chalcospilans* Dyar. Another station was established on Flamenco Island, one of the three small islands at the Pacific entrance to the Panama Canal, to obtain information on the habits of *Haemagogus argyromeris* Dyar and Ludlow. No platforms were built at these two stations, at Old Panama and Flamenco Island, but biting collections were made at ground level one day during each week.

During 1950, the second year of this work, 38 tree stations, consisting of a single platform each, were established and operated during the rainy season, throughout eastern and western Panama. These stations were located in Darien and Colon Provinces, east of the Panama Canal, and in Panama, Cocle, Herrera, Veraguas and Chiriqui Provinces west of the Canal Zone. One of the writers, Carpenter, went to Costa Rica in May, 1950 and set up four tree stations in Puntarenas Province, two each at Esquinas and Jalaca. These stations in Costa Rica were operated with the assistance of personnel of the Golfito Division of the United Fruit Company. The presence of *H. spegazzinii falco* and *A. leucocelaenus* in Costa Rica was definitely established during this work in 1950, even though the Costa Rican stations were operated for only a short period (Galindo *et al.,* 1951b). Hourly catches, from 9:00 a.m. to 3:00 p.m. one day each week, were made of mosquitoes approaching the collectors to bite at both ground and canopy levels at each of the tree stations operated during 1950.

In April, 1951, one of the natives in the group surveying for the right-of-way for a road from Almirante to Boquete in Northwestern Panama died in the Almirante Hospital of yellow fever. Four tree stations were immediately established in this area in northwestern Panama in Bocus del Toro Province and our catches from these stations yielded more *H. spegazzinii falco* than we had obtained any place in Panama, except in Cocle Province in midwestern Panama.

Later during the summer, 1951, there were reports of sylvan yellow fever in Costa Rica, particularly in Limon Province, adjacent to Bocus del Toro Province of Panama. The disease has since

Fig. 4. Bamboo section larval trap used for studying the breeding habits of mosquitoes in the forest.

Fig. 5. Bamboo larval trap with a cover for the large opening in the top. The hollow bamboo section is made accessible to mosquitoes by means of a small opening cut through the side.

occurred in other provinces in Costa Rica. During July, August and September, 1951, there were over 170 cases diagnosed as yellow fever in Costa Rica, with between 30 and 40 deaths.

Two of the writers, Galindo and Trapido, went to Costa Rica at the beginning of August, 1951, and set up four tree stations at two places (Wauchope, in Limon Province, and at San Gerardo in Alajuela Province, north of San Jose) in an attempt to get mosquitoes through to Panama for recovery of virus. The shipments of live mosquitoes were usually delayed in reaching the laboratory and virus was not recovered. However, we were able to get considerable information on the mosquito fauna of the forest canopy in these areas during August and September, 1951.

DISCUSSION OF SPECIES. Three species of *Haemagogus, H. spegazzinii falco, H. equinus* Theobald and *H. lucifer* Howard, Dyar and Knab are generally abundant and widely distributed in the forests of Panama and Costa Rica. Three other species, *H. argyromeris* Dyar and Ludlow, *H. chalcospilans* Dyar and *H. iridicolor* Dyar are less abundant or are more limited in their distributions.

Haemagogus spegazzinii falco, the principal known vector of sylvan yellow fever in northern South America, is found in the virgin rain forests, particularly on the slopes of the mountains, throughout much of central and western Panama and Costa Rica. The females are predominantly arboreal and are avid feeders on man under these conditions.

During these studies, we observed that this species tends to have a spotty distribution within the forest. For example, one tree station in a given area would often yield a large catch, while a nearby tree station would be negative for the same period. It may be that the activities of the females are largely confined to areas of the forest where optimum conditions exist for such activity; however, we were not able to recognize any significant differences in either of these environments. The males of this species were never encountered in the forest, and we were able to get males only by rearing larvae or by obtaining eggs from gravid females and hatching and rearing the larvae.

Although the larvae were found in tree-holes and in bamboo traps on several occasions, we were never able to find them in numbers commensurate to the number of adults found in the area. For a while, we were of the opinion that the larvae might be more abundant in rot cavities high up in the trees, in the forest canopy. During 1950, a large series of tree-holes were located in the forest canopy by climbing, and were subsequently examined each week over a period of two and one-half months for larvae. Larvae were not any more abundant in these tree cavities in the forest canopy than in bamboo traps situated in similar locations.

It was interesting to note that the eggs of *H. spegazzinii falco* apparently do not begin hatching until after at least two or more floodings and drying of their breeding places. The adults appear much later in the forest after the beginning of the rainy season than do other *Haemagogus* spp. The maximum number of larvae found in a breeding container during a single examination was seldom more than four or five. The fact that the eggs do not all hatch following a single flooding, insures the species against adversity.

Haemagogus spegazzinii falco was particularly abundant at our Pacora stations in central Panama where the outbreak of sylvan yellow fever occurred in January 1949. Our catches also showed heavy populations in Cocle Province in midwestern Panama, at Almirante in Bocus del Toro Province in Northwestern Panama, where yellow fever occurred in May, 1951, and in Costa Rica at Wauchope in Limon Province and at San Gerardo in Alajuela Province, north of San José. At Almirante in northwestern Panama, the total catch of *H. spegazzinii falco* was almost as great as the combined total of *H. equinus* and *H. lucifer*. At Wauchope in Costa Rica, where a large number of cases of yellow fever occurred in humans, *H. spegazzinii falco* outnumbered all other *Haemagogus*.

Haemagogus equinus and *H. lucifer* are also found mostly in the deep virgin forests and also feed in greater numbers in

the canopy of the forest than at ground level. These species are widespread throughout the area studied, and are generally more abundant than *H. spegazzinii falco* in many areas. The larvae of both species, *H. equinus* and *H. lucifer,* were plentiful in tree-holes and bamboo traps during the rainy season. *Haemagogus equinus* was particularly addicted to breeding in bamboo traps, more so than any other *Haemagogus* spp. found in the area.

The mating of males and females of both *H. equinus* and *H. lucifer* was observed by the writers on several occasions. The males were attracted to the collector in the forest and remained in flight a distance of three or four feet away, until the females arrived to feed. As the females approached the collector, they were caught by the males and copulation took place during flight.

Other *Haemagogus* spp. occurring here are either less numerous or are more restricted in their distribution. *Haemagogus argyromeris* is generally found in or near second growth timber and rarely invades the deep virgin forest. *Haemagogus chalcospilans* breeds mostly in rot cavities in mangrove trees found in the tidal marshes.

Aedes leucocelaenus, a known vector of sylvan yellow fever in northern South America, but generally of lesser importance than *H. spegazzinii falco,* is a prominent member of the mosquito fauna of the heavy virgin forests in this area. The females of *A. leucocelaenus* feed at ground level and in the forest canopy, appearing to favor the canopy for feeding in many areas.

Other members of the subgenus *Finlaya* commonly occurring in the forests in Panama are *A. leucotaeniatus* Komp and *A. terrens* (Walker). One member of the subgenus *Howardina, A. quadrivittatus* (Coquillett) which occurs in western Panama displayed a distinct preference for feeding in the forest canopy.

Members of the genus *Sabethes* may be regarded with some suspicion as possible vectors of sylvan yellow fever, since they appear to be the only diurnal, canopy feeding mosquitoes remaining in the forests in numbers during most of the dry season, when *Haemagogus* spp. and *Aedes* (*Finlaya*) spp. have practically disappeared as adults.

Sabethes chloropterus is predominantly a canopy-feeding mosquito and is widely distributed and abundant in many areas in Panama and Costa Rica. *Sabethes cyaneus* and *S. tarsopus* Dyar and Knab are somewhat less common than *S. chloropterus* but likewise show a preference for feeding in the forest canopy. The larvae of *Sabethes* develop in water in deep tree-holes having small openings. The larvae of two of the species, *S. cyaneus* and *S. chloropterus,* readily took to breeding in bamboo traps which had the top closed and a small opening cut through the side, simulating their natural breeding places.

One member of the genus *Trichoprosopon, T. magnum* (Theobald), which breeds in water collections found in the leaf axils of plants, proved to be predominantly arboreal in its feeding; however, the species has a rather limited distribution in the area.

At some of our lower stations, pest species, particularly *Aedes* and *Mansonia,* were very annoying to our collectors situated at ground level during certain seasons of the year. At one station, located near extensive salt marshes, *A. taeniorhynchus* was at times so abundant that it was almost unbearable for the collector situated on the ground, yet this mosquito was rarely encountered by the collector situated on a platform only twenty feet above the ground.

SUMMARY. A study of the distribution, occurrence and habits of diurnal forest mosquitoes in Panama and Costa Rica is described, following an outbreak of sylvan yellow fever which was first recognized in Panama in January, 1949.

Adult mosquitoes were captured in the forest at ground level and also in the forest canopy, where human collectors were stationed on platforms reached by ladders. Larvae were collected regularly in the forest from tree-holes and bamboo larval traps.

Pertinent information is given on outbreaks of sylvan yellow fever occurring in

central Panama during 1949 and in northwestern Panama and Costa Rica during 1951.

The habits of common species of canopy-feeding mosquitoes of this area, belonging to the genera *Haemagogus, Aedes* (*Finlaya*), *Sabethes* and *Trichoprosopon,* are described.

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MOSQUITO CONTROL IN THE SOUTH PACIFIC

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A trip to Tahiti to attend a conference on filariasis provided a good opportunity to observe the mosquito situation on some islands in the Pacific. Because mosquito-borne diseases are a major health problem and because of fast air transportation, the possibility of these diseases spreading from these islands to Hawaii necessitates constant vigilance in our control program.

Both filariasis and dengue are common in many islands of the South Pacific. It is because of the wide prevalence of filariasis and the importance of its control, that the South Pacific Commission held a conference on this disease to improve the health of this part of the world.

Tahiti was chosen for the site of the conference because an active program of filariasis control is being carried on there. Intensive research on filariasis is being conducted by the Institute of Medical Research of French Oceania located on the island of Tahiti with headquarters in Papeete, the capital. The Institute is housed in a well-equipped building, with a staff of twenty-seven persons. French and American scientists cooperate in the work of the Institute in solving the problem of filariasis control.

The principal vector of filariasis in the South Pacific is *Aedes pseudoscutellaris.* Its control is complicated by the many empty coconut shells lying around the plantations which furnish suitable breeding places for this mosquito. As the production of copra is an important industry on these islands, coconut plantations are numerous.

Mosquito control inspectors are attempting to educate the planters not to leave empty coconut shells on the ground but to assemble them into piles and to destroy them by burning. Another mosquito control measure around the house and on the plantations consists of keeping down weeds and shrubbery, as the vector mosquito has

the habit of biting outdoors among such vegetation.

Along with the day biting *Aedes,* the night mosquito, *Culex quinquefasciatus* was also incriminated in Tahiti as a possible vector of filariasis. This confirms our findings in Hawaii that the same local night mosquito here may be a suitable vector of the non-periodic form of filaria parasite prevalent in this part of the world.

Spraying of houses and the surrounding shrubbery with DDT as well as minor drainage is being carried on by the mosquito control teams on Tahiti.

The mosquito control program in Fiji is well organized, with about 75 inspectors covering the islands. House-to-house inspection is carried on in the city of Suva as well as in other centers of population. The inspectors are well trained and the public is fully aware of the danger of mosquito-borne diseases.

Fiji is so mosquito-conscious that an anti-malaria program is underway even though no *Anopheles* has yet been established on the islands. The Sanitation Division of the Fijian Medical Department has built concrete storm drains in and around their cities and towns so that the *Anopheles* will have less available breeding places in case any of them should enter the islands. The control of pest mosquitoes is also an integral part of community sanitation.

The areas with malaria-carrying *Anopheles* closest to Fiji consist of New Hebrides and Australia. The airport of Nandi and the seaport of Suva on Fiji are closely guarded against the introduction of *Anopheles* from these regions.

It was of interest to know that *Megarhinus splendens,* a predacious mosquito introduced into Fiji from Java in 1931, has established itself and has spread from the places where it has been released. Studies are being carried on to determine its part in keeping down the spread of filariasis. An insectary will also be constructed in Suva to ascertain if this species will mate indoors, as does the South African species which we have been propagating in Hawaii.

The control of mosquito-borne diseases on neighboring islands of the Pacific, which are drawn closer and closer to Hawaii by the ever increasing speed of modern air transportation, is of vital interest to our Territory, as well as on the mainland of America. Our mosquito control program also concerns their welfare. Any progress made on different parts of the Pacific area will be of mutual benefit in this shrinking world of ours.

There are a few imported cases of filariasis in Hawaii. They are among new immigrants to the Territory from South Pacific islands. So far, we found 12 out of 186 of these to be carriers of microfilariae in their blood. These positive cases were only found among those who have been in Hawaii for less than six years. The microfilariae evidently disappeared after a longer period of residence in the absence of reinfection.

Infection experiments which we carried on with these South Pacific islanders as a source of infection showed that our night mosquito, *Culex quinquefasciatus,* could act as a suitable intermediate host for the non-periodic form of *Wuchereria bancrofti.* Transmission of filariasis in Hawaii appears not to have taken place as no infection has been found yet among those born and reared in the Territory. However, constant vigilance in checking the infection and in mosquito control measures need to be exercised to prevent the establishment of this disease so common in other Pacific islands from gaining a foothold in Hawaii.

The dengue epidemic which Hawaii suffered in 1943–44 was likely to have been imported from the South Pacific. This supposition is primarily based on the knowledge of the cases of two airline pilots who were hospitalized with dengue in Honolulu earlier in July, 1943, after arrival from Suva, Fiji Islands, where an epidemic of dengue had been reported. One of the pilots was already ill when he came to Honolulu, but the other had onset of symptoms several days later and was not isolated by hospitalization until he had passed through the infectious period. The

fliers occupied an apartment at a Waikiki rooming house. The maids at this house later came down with what was subsequently suspected of being dengue. By August 8, 1943, Waikiki had become such a focus of infection that it was restricted to military personnel.

As dengue is common in South Pacific islands, there is always the danger of its introduction again into Hawaii. There are two commercial airlines connecting Honolulu and Australia, stopping over on Canton Island and Fiji Island. The Philippines and the Orient are also endemic regions for dengue. The day mosquito control program in Honolulu is a preventive measure to keep the dengue vector population at such a low level that an epidemic may not occur again in case dengue should enter into this crossroad of the Pacific.

Airlines crisscrossing the Pacific have reduced distances in term of flying hours. One can now reach Australia in less time by plane from Honolulu than it takes to make an inter-island trip on boat to the port of Hilo on one of the neighboring islands within our Territory. The South Pacific is not any more a remote spot far away from the rest of the world. It will be still closer as connecting airlines link some of the more remote islands to major airports.

The South Pacific Commission is actively attacking the program of filariasis and of mosquito control as part of its health activities. Along with the researches and field studies carried on by the Institute of Medical Research in Tahiti, the joint anti-filariasis campaign is already showing good progress in the control of this mosquito-borne disease in the South Pacific.

REPORT OF THE A.M.C.A. COMMITTEE ON RESEARCH AND DEVELOPMENT OF MOSQUITO CONTROL AND RELATED PROBLEMS

Assembled by
B. B. PEPPER

INTRODUCTION

At the direction of President Don Rees, there was appointed a special committee on research and development of mosquito control and related problems. The members of this committee were as follows:

Harold F. Gray, Alameda Co. Mosquito Abatement District, Oakland, California.

William E. Bickley, University of Maryland, College Park, Maryland.

J. A. Mulrennan, Florida State Board of Health, Department of Entomology, Jacksonville, Florida.

B. V. Travis, New York State College of Agriculture at Cornell University, Ithaca, N. Y.

B. B. Pepper, New Jersey Agricultural Experiment Station, Rutgers University, New Brunswick, N. J., Chairman of Committee.

The purpose of this committee was threefold. First, to select some of the more important contributions from the current literature and to suggest the possible significance or application of this information. Second, to prepare a list of some of the more important problems confronting mosquito control workers at present, that should be solved to implement control work. And third, to include other pertinent information which the committee felt would assist mosquito control workers.

The committee was given a free hand on the development of its report. An outline

was prepared and the members of the committee each prepared a section of the material.

It was the opinion of this group that the report should not be a review of the literature or a detailed report of mosquito work during 1951, since these topics are covered by Dr. F. C. Bishopp, and others in their "Review of Contributions to World Mosquito Problems" published annually in the Proceedings of the New Jersey Mosquito Extermination Association and by Dr. H. H. Stage and H. Sollers in their "References to Literature of Interest to Mosquito Control Workers" published in *Mosquito News*. Therefore only a brief summary of the current work will be given. Suggestions on some of the problems needing attention are listed.

The committee wishes to express its appreciation for the cooperation of Drs. Bishopp, Stage and Knipling in supplying abstracts of current literature and for valuable suggestions. The committee is also indebted to Drs. Nielsen and Provost for their excellent outline which is appended to this report.

Significant contributions to knowledge of mosquitoes and mosquito control in 1951

Taxonomy and Morphology

It is often difficult for practical mosquito control workers to see how fundamental research in general and taxonomic and morphological studies in particular will have any relation to their immediate problems. Indeed there are instances in which an outstanding piece of work can have no claims toward practical application, but no apologies need be offered. This section of the report, instead of describing contributions to knowledge in 1951, will refer to a few examples of research work and attempt to indicate actual or potential values to those engaged in mosquito control operations.

A thorough study of culicine pupae of the Northeastern United States now makes it possible to identify these mosquitoes in the pupal stage. If certain species in a given area do not need to be controlled, this makes greater selectivity possible. Similarly a method, described in 1951, for identifying the early stages of some anopheline larvae is useful in a "species sanitation" program. Investigations dealing with the systematics of the subgenus, *Melanoconion* (genus, *Culex*) in Central America and Northern South America, studies of the *Aedes punctor* subgroup in the North American Arctic, and additions to our knowledge of *Aedes* mosquitoes in Texas and the American tropics are extremely worthwhile for those who must survey and plan control work in those areas.

Biology and Ecology

All investigations which increase our understanding of mosquito biology and ecology are important in the evaluation of the relative importance of different species as pests or as disease vectors. Several outstanding studies of flight range and dispersal were reported in 1951. Some of these made use of radioactive isotopes. It is now possible to make adult mosquitoes radioactive by feeding them aqueous solutions of P^{32}.

The number of broods of three species of *Aedes* in California rice fields was observed, and this information should have direct application in control work. Likewise a host of new data was obtained relative to the ecology of mosquitoes breeding in irrigated pastures in California.

It was reported that dipping may be of uncertain value as a technique for establishing the presence of anopheline larvae when populations are very low. A study of the mechanism involved in floating anopheline larvae at a water surface showed that two ear-like structures on the thorax enable the larvae to float the anterior end of the body and indicated that the palmate hairs are auxiliary floating structures. This has implications in the direction of the development of new larvicides. Much progress was reported in 1951 on some basic questions regarding responses of female *Aedes* mosquitoes to water va-

por, carbon dioxide, chemical compounds found in body exudations, temperature, and colors. It seems obvious that investigations on attractants have practical value and that the results are especially useful in furthering the search for better repellents.

Studies of the behavior of certain species of *Anopheles* have been reported. These contribute to an understanding of the factors involved in the development of resistance to DDT and other insecticides as well as help in increasing the effectiveness of insecticidal applications. Experiments were conducted to determine the hazards to wildlife when DDT was used to control mosquitoes on a New Jersey salt marsh. —W. E. BICKLEY.

DISEASE TRANSMISSION

The year 1951 represents roughly the fifth year since DDT became generally available for mosquito control throughout the world. Because of the unique susceptibility of *Anopheles* to this insecticide, especially when it is used as a residual deposit of considerable effective duration on surfaces within dwellings, there has generally been a dramatic and rapidly accelerated reduction in malaria in those areas where it has been extensively applied. At the present time well over fifty millions of people in various parts of the world are largely dependent on an adequate supply of DDT (or insecticides having a similar effect) for their working capacity and even life.

But it would appear that too great a pre-occupation with the problem of malaria eradication via the DDT residual spray route may lead to a possible future disaster, in view of the present unsettled conditions of the world. Shipments of adequate amounts of insecticides could be interrupted by war. Some thought should be given to alternative control methods, including the reduction of larval habitats, and the effective use of alternative materials as larvicides. An enforced return to either natural pyrethrins, paris green or petroleum derivative oils is not inconceivable. It may also later on prove to be a mistake to have abandoned the concept of screening as a malaria control measure.

Since it is possible that the anophelines may yet develop the degree of resistance to DDT and its analogs which has appeared in a number of *Culex* and *Aedes* species, a degree of caution should pervade any predictions as to the possibilities of extirpation of mosquito-transmitted diseases in tropical areas. Two possibilities can be foreseen: (1) the development of resistance to insecticides by the vectors; (2) due to unstable world conditions any large scale war may interrupt the delivery of necessary insecticides to large areas of the world. The present preoccupation by many workers with insecticidal measures as the sole control method could lead to disastrous results in such cases.

Even the concept of complete extirpation of an arthropod-transmitted disease, now seen to be an actual fact or a definite possibility within a few years in circumscribed areas, may need qualification. As long as all goes well, extirpation is a definite goal of great promise, but what if a war interrupts operations? A population no longer tolerant to a specific disease will have been developed, and the re-introduction of the disease and its vectors from outside areas could easily end in an enormous epidemic in a non-resistant population.

On the other hand, the use of native resources in labor and materials, for drainage, clearing, stream flushing, shading, salinification, and other well established control measures, in conjunction with insecticides as needed, would in the case of interruption of delivery of insecticides, or in the case of development of vector resistance to insecticides, leave the human populations in tropical areas with at least some means of protection, which could be intensified to whatever degree is necessary to minimize disease incidence.

We should also recognize that while we have (at present) a very potent weapon in DDT and its analogs, nevertheless malaria has been extirpated from extensive areas of the world without any conscious attack on the vector, and in fact in the continued

presence of at least moderate numbers of the vectors (Upper Mississippi, Ohio and Missouri River Valleys). In the last two hundred years *Aedes aegypti* and yellow fever have disappeared from the north Atlantic coastal area of this continent, even though Halifax was formerly its apparent northern limit at the time. Is it not possible that we have not fully studied these unfavorable changes for either the vector or the parasite? Could not more be learned and applied concerning the modification of the environment for the purpose of disease prevention?

But, irrespective of the long-range viewpoint, spectacular results in the control of mosquito-transmitted disease continue to be reported. Giglioli reports the virtual extinction of *Anopheles darlingi* and the almost complete eradication of malaria in British Guiana, by means of residual DDT spraying of dwellings as the only control measure. Gabaldon reports on the great reduction in malaria in Venezuela with a concurrent reduction in the general death rate and an increase in the birth rate in the past five years. Pinotti also reported that in Brazil the operations of the National Malaria Service have been so encouraging that eradication of malaria from Brazil may be achieved eventually.

Rao has reported on an interesting facet of control in the Eastern Province of Afghanistan. Malaria was effectively controlled in a hyperendemic area by residual DDT sprays in the houses, even though the population largely slept out doors. The local vectors are *Anopheles culicifacies* and *A. superpictus*.

Downs and Bordas have also reported excellent results in the endemic area of Xochimilco-Mixquic at an elevation of 7400 feet in the Valley of Mexico, using DDT residual sprays. Parasite rates have dropped to nothing and the spleen rates were greatly reduced.

An important conference on malaria in Africa began on November 30, 1950 at Kampala, Uganda. This was reported in Technical Report Series No. 38, "Malaria Conference in Equatorial Africa," by WHO in April, 1951. Among the subjects discussed by some sixty experts from various parts of the world were: the distribution and prevalence of malaria in Africa; the species of *Plasmodia;* the species of vectors; the economic importance of malaria; and methods for its control.

A valuable tool in the diagnosis of malaria (Nat. Inst. Health Bul. 180—Microscopical Diagnosis of Malaria) has been revised and brought out as a second edition.

Considerable work continues to be done on the plasmodia of birds, rodents and monkeys. Although not of immediate practical applicability, the study of these parasites and their relations to both host and vector may ultimately produce data of fundamental value in human malaria. Such studies deserve continuance and encouragement.

The problem of myxomatosis in rabbits continues to excite interest in Australia. The mosquito vectors appear to be fairly well defined, but there is a definite question as to whether this disease can be safely encouraged as a means of reducing the rabbit population, if there is a chance that virus encephalitis may be increased in the human and equine population. A report from W. C. Reeves on this problem is expected in 1952.

Additional experiments tend to further incriminate *Culex tritaeniorhynchus* as a vector of Japanese B encephalitis, but there appear to be many factors in the transmission of the disease still unsolved.

Very little of significance as to either yellow fever or filariasis occurred during 1951.

Research is particularly needed in the field of insect physiology, partly to explain the mechanism of DDT resistance in general, and also to explain the difference in the rate of acquisition of resistance between *Culex* and *Aedes* species on the one hand, and *Anopheles* on the other. In the long run we may find that true research in general insect physiology, as contrasted with technical investigations directed only against the immediate problem, may prove to be more valuable in providing an ultimate answer to some of the present problems.

In the field of chemistry there appears

to be an opportunity for the development of a potent insecticide which is radically different in its chemical structure from the chlorinated hydrocarbon group of which DDT is a type, and with much less toxicity to warm blooded animals than the organic phosphate group (Parathion, etc.). Such an insecticide could be substituted for the DDT group when resistance to the latter has developed to an appreciable degree.—HAROLD F. GRAY.

Significant Contributions to the Chemical Control of Mosquitoes in 1951

Numerous papers were published during 1951 that presented useful information on the chemical control of mosquitoes. Any attempt to summarize such a large amount of data must, because of space limitations, omit some rather significant papers and details. This summary will include only the references in which results are given on new and uncommonly used techniques, equipment, or materials. Also data will be included for species for which information is either meager or not in the literature.

LARVICIDES: Immediate Control.—Ginsburg compared allethrin and pyrethrins in both colloidal water solutions and in oil emulsions with laboratory tests against *Aedes aegypti*. He found that against larvae the pyrethrins were two or more times more toxic than allethrin. He noted no material increase in the toxicity of allethrin when synergists were added. Kinsey with field tests in New York State found that it was necessary to apply DDT in oil or in emulsion form weekly to polluted water around a public dump to control the *Culex* larvae. One treatment with chlordane emulsion lasted three months and two treatments lasted from May to October. Trapido made field tests with *Anopheles claviger* in Sardinia and the mainland of Italy. He found that the amount of DDT required to kill 95 per cent or better of the larvae was one part in 10 million parts of water or about one-twentieth the amount needed for *Anopheles quadrimaculatus*. He also found that after about four years of intensive treatments in Sardinia that *A. claviger* was no more resistant to DDT than this same species from the Italian mainland.

Soroker made a series of tests against *Culiseta incidens* with emulsions of a number of the newer organic insecticides. Heptachlor, aldrin, parathion and a DDT emulsion with Lethane 384 used as a solvent were all superior to DDT.

REPELLENTS: Skin applications.—Applewhite and Cross presented data to show that dimethyl carbonate and propyl N,N-diethyl succinamate were the most effective of a series of repellents tested in Alaska. Travis presented laboratory tests on the 30 most effective repellents out of 4,313 tested against *Anopheles quadrimaculatus*. This anopheline was difficult to repel. Only four materials, p-isopropoxybenzaldehyde, alpha-amylcinnamaldehyde, methyl beta-diethylaminocrotonate and N-amyl succinimide were effective for as long as three hours. Dimethyl phthalate was the only one of the commercially available repellents that was sufficiently effective to be included with the top 142 materials. *A. punctipennis* and *A. freeborni* were repelled for much longer times, and *A. gambiae, A. albimanus* and *A. aquasalis* for shorter times, than *A. quadrimaculatus,* with the same materials. Travis and Smith summarized the available data on 14 repellents and on 12 mixtures that are deemed safe to use on man. Data are presented for several *Aedes* species and for *Anopheles quadrimaculatus*. The three most promising chemicals against *Aedes* species were propyl N,N-diethyl succinamate, Repellent 6–12, and dimethyl carbonate. Against *Anopheles quadrimaculatus* only dimethyl phthalate was outstanding.

Clothing applications.—Smith and Cole conducted laboratory tests against *Aedes aegypti* and *Anopheles quadrimaculatus,* and field tests against *A. taeniorhynchus* and *A. sollicitans*. In the laboratory 2,4-nonanediol, N-butyl-1,2,3,6-tetrahydrophthalimide, 2-butyl-2-ethyl-1,3-propanediol, dimethyl phthalate and Repellent 6–12 were effective 8 to 20 days. The best material, mixture M-1960, was effective for

30 to 31 days. The mixture contained equal parts of 2-butyl-2-ethyl-1,3-propanediol, N-butyl-acetanilide and benzoate. Applewhite and Cross found that in Alaska their most effective material was 2-[2-(2-ethylhexyloxy)-ethoxy] ethanol.

Special studies.—Sarkaria and Brown describe a technique for testing repellents against mosquitoes by an olfactometer method. The method was designed to test the correlation of vapor pressure with repellency. It was concluded that repellency of compounds could not always be correlated with vapor pressure.—B. V. Travis

Control—Adult, Larvae and Pupae

Biological Control. The annals of mosquito control are well documented concerning the value of surface feeding fish as predators of mosquito larvae. Of the large number of invertebrate parasites and predators of mosquitoes relatively few reports are available. It is well known that many insects, mites, nematodes, coelenterates, and protozoa attack mosquitoes. However, only in a few cases are the parasite or predator-host relationships fully understood. Perhaps equally important and even less understood are the diseases of mosquitoes caused by fungi, bacteria, and viruses. Little has been published on biological agents during the past several years.

Physical Control: Water Management. In view of the fact that some species of mosquitoes are showing resistance to the chlorinated hydrocarbon insecticides, water management may regain the popularity it held prior to the advent of DDT. One of the most thorough evaluations of water management and the ramifications connected therewith presented in 1951, was given in a symposium in the Proceedings of the 38th Annual Meeting of the New Jersey Mosquito Extermination Association. The symposium consisted of the following: "Introductory Statement," by L. A. Stearns; "The Relationships of Water Management and Drainage to Other Forms of Mosquito Control," by D. M. Jobbins; "Water Management and Drainage in Open Salt Marsh Areas for Mosquito Breeding Control," by Fred A. Reiley; "Mosquito Control on An Enclosed Salt Marsh," by George E. Powers; "Fresh Water Swamps with Reference to Surface Drainage," by Robert L. Vannote; "Water Level Management for the Control of *Anopheles quadrimaculatus* in the Tennessee Valley," by T. F. Hall.

Each participant in the symposium reviewed the particular problems involved in his area. In summarizing the entire discussion on water management, it becomes apparent that when properly done, it destroys mosquito breeding in areas where other less permanent measures are impractical or too costly. It was also emphasized that installations for controlling water if properly planned and executed will last for considerable periods of time.

After a few observations of the hazardous effects of chemicals, one realizes the importance of water management on the preservation of the natural environment.

General. a. Organization and Administration. During the past year several papers were presented on the organization of mosquito control or abatement districts. The general tone of these discussions was the re-evaluation of the organizational setup. The legal obligations of a mosquito control commission or abatement district were stressed.

b. Limitations in Use of Methods. Economics appears to be the chief limiting factor in the prosecution of control activities based on many different reports from the various sections of the country. High labor and material costs have made it necessary for many agencies to adopt less costly methods at the sacrifice of efficiency. In some areas of the country, labor was exceedingly scarce. In order to carry out the bare essentials unskilled and inexperienced labor had to be used. This has apparently led to more mechanization with simplified equipment.

c. Hazards. 1. Wild Life. Although it has been recognized that the synthetic organic insecticides presented a hazard to certain forms of wild life, most of the re-

search has been associated with agricultural and forestry operations. In 1951, Springer and Webster (Mosq. News Vol. II, pp. 67–74) report large scale experiments conducted on salt marsh areas in which varying dosages of DDT were used. In general, their studies show that considerable damage to fish and crabs resulted at dosages of 0.5 pounds or more per acre. Birds showed little or no direct effects of the spray.—BAILEY B. PEPPER.

Outline of Research Needed in Advancing Knowledge of Mosquitoes and Their Control. (Biology)

BIOLOGY SUBDIVISIONS.—a. *Ethology:* life history under natural conditions. 1. Habits of post-embryonic stages: eggs, larvae, pupae. 2. Habits of adults: emergence, phases of behavior at different adult ages, active and passive periods, resting places, copulation, migration, swarming, food (bloodmeal and other natural food), longevity, oviposition.

b. *Ecology:* the animal in its relation to the environment. 1. Synecology: as a part of a biocoenose. 2. Autecology: as a reactor to external factors. Simultaneous studies of microclimatic conditions in the habitat and reactions in the laboratory of all stages; main factors to consider—temperature, humidity (eggs and adults), salinity, pH, food, parasites, predators, population studies. Preference: activity as influenced by different factors.

c. *Physiology:* the understanding of the biological mechanisms of the animals. 1. General indicator: respiratory metabolism. 2. Biochemical aspect: osmoregulation of aquatic stages, digestion, enzymatic and hormonal influence, reproduction, ecdysis, diapause. 3. Reflex biology: taxis, basic reflexes, chain reflexes, "Instinct-Dressur-Verschrankung," appetite behavior, plasticity.

d. *Technique:* proper methods and tools. Collecting, sampling, microclimatological methods, physiological methods, statistics.

EXAMPLES. Any subject could be approached in all four (a to d) ways, above, e.g.: 1. A student of the larval biology of *Aedes sollicitans* may work out actual findings in the field (a), mortality and length of stages at different salinities (b), the osmoregulatory mechanisms (c), or find a new method to determine chloride content of water (d).

2. An investigation of resting places of *Aedes taeniorhynchus* would produce findings elucidating the general ethology of mosquitoes (a), microclimatic factors in autecology (b), possible metabolic rhythms (c), and probably giving a clue to new sampling techniques (d).

3. A study of the food habits of *Mansonia perturbans* would throw light on activity and flight rhythms (a), the relation of adults of this species to the flora as a possible direct source of food or as a habitat for warm-blooded vertebrates yielding blood food (b), the role of metabolism in orienting the movements of the adults (c), and conceivably a new statistical approach to the problem of establishing true food preferences (d).

4. The establishment of an insectary colony of *Psorophora confinnis,* or any species for that matter, would assuredly yield much biological information beyond merely adding new techniques. Almost all subdivisions of the outline could be advanced by the proper interpretation of observation incident to the successful establishment of such a colony.

(Examples of problems)

A. TAXONOMY AND MORPHOLOGY.—Problem: Search for improved method of separating *Culex* group. Definition of complexes if such exist. Ex. *pipiens—salinarius.*

B. BIOLOGY AND ECOLOGY.—Problems: (1) Thorough study of U. S. eastern *Culex.* Biting habits, necessity of blood meal for reproduction. Influence of humidity and temperature on movement and urge to enter dwellings. (2) Clarification of alleged ability of *Aedes cantator* to breed in fresh water. (3) Biology of mosquito ova. Study of conditions required for hatching eggs of salt marsh *Aedes* species. (4) Flood water mosquitoes—oviposition site preferences. Why, if any?

Are entire marshes seeded for margins only? Do salt marsh margins produce heavily because conditions for hatching are more frequently favorable? Factors affecting selection of oviposition site of *Wyeomyia smithii*. (5) *Mansonia perturbans*. Biology with reference to distributions in a "favorable" marsh; attachment preferences of larvae; larval food preferences; larval migration; completion of general life history, especially chronological development of overwintering larval stages. (6) The relationship between mosquito habits and the development of insecticide "resistance," resting and flight habits with regard to accessibility of mosquitoes to insecticide applications. (7) Techniques—colony rearing of *Aedes sollicitans* and other species in study of biology and potential disease vector significance.

C. MOSQUITOES AS DISEASE VECTORS.—Problem: Survey of *Aedes sollicitans* and *Culex pipiens* and other common species for presence of certain viruses.

D. CONTROL.—Problems: (1) Mosquito ovicides, an open field. (2) Study of the effect of certain insecticides applied for mosquito control on plankton and food organisms of mosquito larvae and associated aquatic life. (3) Chemical residues in soil of areas treated to suppress mosquito breeding and their effect on the biological balance of marshes. (4) Further observations on the effect of chemicals used for mosquito control on wildlife, fish, birds, shellfish. (5) Insecticide "resistance." Is rotation of dissimilar chemical toxicants in formulations an answer? (6) A reexamination of predaceous fishes and their management for mosquito control on tidewater meadows. (7) New sampling procedures and equipment for evaluating mosquito prevalence and movement. (8) Reexamination of the potential use of fungi, bacteria, etc., in the control of mosquitoes. (9) Many equipment needs—and exploitation of possible techniques developed for related fields, etc., etc.—ERIK TETENS NIELSEN AND MAURICE W. PROVOST.

All persons interested in mosquitoes and their control should have a reference copy of AMCA Bulletin No. 2, "Ground Equipment and Insecticides for Mosquito Control" before the start of another season. Order from Mr. C. T. Williamson, Suffolk Co. Mosquito Control Commission, Yaphank, N. Y. Price $2.00.

See page 219 for information on next annual meeting, to be held at Daytona Beach, Florida, in April.

ARTICLES

THE DISPERSAL OF *AEDES TAENIORHYNCHUS* I. PRELIMINARY STUDIES

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INTRODUCTION

The salt-marsh mosquitoes, *Aedes taeniorhynchus* (Wiedemann) and *A. sollicitans* (Walker), are among Florida's worst insect pests. They have been fought by organized districts since 1927, and in 1951 an estimated million and a half dollars were spent on their control. The biology of these mosquitoes, nevertheless, is largely unknown, particularly in their adult phase. Our dispersal studies are aimed not merely at establishing a "flight range" but more especially at elucidating the mechanics of such flights. The best hope for efficient control of these far-ranging insects lies in a thorough synthesis of their ecology, physiology, and group behavior. Concurrent research in all three of these phases is being carried on by the Division of Entomology of the Florida State Board of Health.

The first dispersion experiment with *Aedes taeniorhynchus* was made in August and September, 1951, on the coast of Lee County in southwestern Florida (Fig. 1). Sanibel is the southernmost of a chain of shell and sand islands extending along the western edge of the shallow bay at the mouths of the Peace and Caloosahatchee Rivers. This bay is roughly 10 by 30 miles and almost in its center is Pine Island, 2 by 15 miles in size, and oriented along the same NW-SE axis. Bay shores in this area are typically mangrove swamps. The outer reef islands, like Sanibel, have mangroves on their bay sides and firm beaches on their Gulf of Mexico or seaward sides. The higher elevations (generally 3 to 10 feet) on the barrier islands are mantled with a scrub of shrubs and low trees. The interior of Sanibel Island has many cordgrass (*Spartina bakeri*) sloughs and cabbage palm (*Sabal Palmetto*) groves. These abundant palms and the plantings of Australian pine (*Casuarina* sp.) constitute the major tree areas of Sanibel's interior. In the northwestern corner of the island there is a large key lime grove. And, of course, the entire bay (north) shore is covered with vast mangrove forests. The islands to the north along the reef—Captiva, Acosta, etc.—are very similar, though much smaller than Sanibel. By contrast, the mid-bay Pine Island is a strip of flat pine-woods much like the nearby mainland. It is completely encircled by mangrove swamps. The mainland on either side of the mouth of the Caloosahatchee River is flat pine-woods with a fringe of mangrove swamp. The bay waters are generally less than 8 feet deep and are sprinkled with mangrove islets. There is, however, a clear expanse of water at the nearest points between Sanibel and Pine Island (2 miles) and between Sanibel and the mainland (2½ miles).

Winds in this area at this time of year (cf. Table I) may be briefly described as follows: (1) pronouncedly eastern and averaging 3–6 m.p.h.; (2) on most days cyclonic disturbances occur in mid or late afternoon, with rain and winds shifting rapidly from all quarters and moving often at speeds up to 15 m.p.h.; (3) by sunset the atmosphere has usually cleared and east winds again prevail, although winds from the other three quarters may assert themselves until well after dark.

The work was centered on Sanibel Island, an area of prodigious *Aedes taeniorhynchus* production. The main objec-

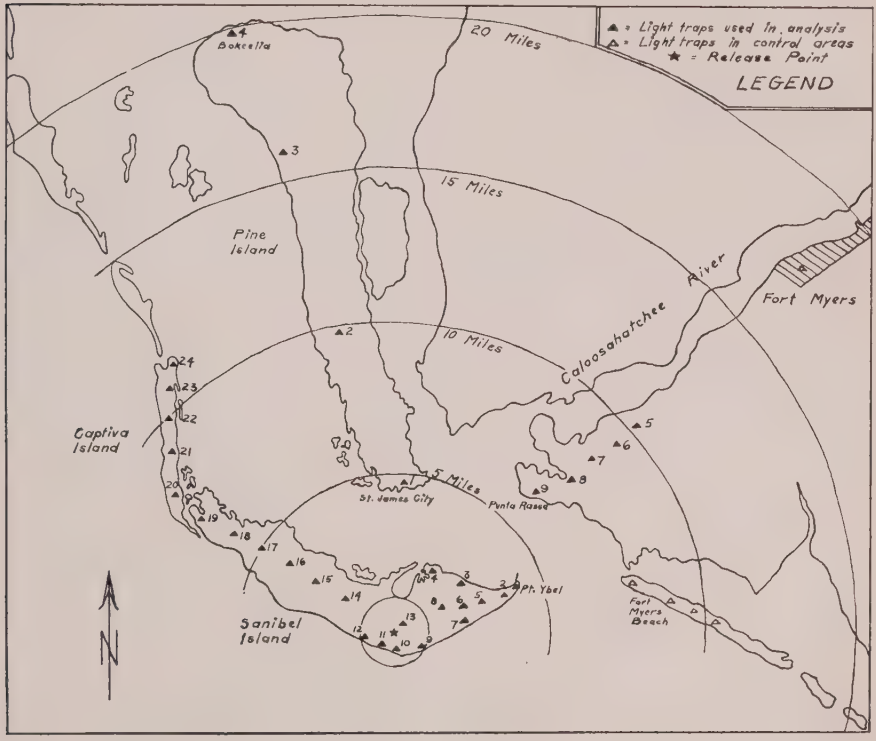

FIG. 1—The San Carlos Bay area, showing locations of light traps with respect to release point on Sanibel Island.

TABLE I. Wind direction at night in San Carlos Bay area at time of dispersal experiment.

Date and hours of night	Number of readings from indicated direction							
	N	NE	E	SE	S	SW	W	NW
	Ft. Myers (U. S. Weather Bureau)							
Aug. 17–Sept. 20								
1800–2400	25	90	131	64	15	29	24	20
2400–0600	4	125	183	61	3	4	2	4
Aug. 19–Aug. 24								
1800–2400	2	18	22	6	4	7	7	0
2400–0600	0	9	45	4	1	0	0	0
	Sanibel Island							
Aug. 19–Aug. 24								
1800–2400	1	3	8	7	1	1	0	0
2400–0600	0	0	1	1	0	0	0	0

tives were to familiarize ourselves with the radioactive isotope marking technique, with the various adult collecting techniques, and with the necessary statistical techniques. The secondary objective was to get some preliminary idea of what to expect by way of dispersion pattern. The technique findings will appear in another paper; the present paper will discuss only the data obtained on dispersion.

Hatching of *Aedes taeniorhynchus* eggs in the San Carlos Bay area is dependent on rains, high tides, or combinations of both. Six large broods occurred in 1951. The first three were strictly tidal in origin and emerged approximately on April 30, May 29, and June 26. Simultaneous heavy rains and high tides, July 18 to 23, gave rise to the largest brood of the year, in early August. Remnants of this brood were still on the wing during the early days of the dispersal experiment. The heavy rains of August 12–13 produced the first major hatch in the interior sloughs of Sanibel Island; this is Brood I of the experiment interval, and the marked mosquitoes were of this hatch. Very heavy rains on Aug. 21–22 produced an even larger hatch in interior sloughs of the entire bay area; this was Brood II of the experiment interval. These two rain-produced broods (I and II) apparently exhausted the supply of conditioned eggs in the entire area, for the torrential rains of mid-September and early October failed to produce any further large broods. The synchrony between the unmarked and marked populations during the experiment is shown in Fig. 2.

METHOD

Larvae were collected from a rain-filled slough and transported in metal drums to wooden tanks at the release point two miles away. The number of larvae in each drum load was computed from 10 ml aliquots. As the four wooden tanks were being filled, larval densities were again computed. Approximately three million third and fourth instar larvae were in the tanks when the isotope was introduced.

The marking technique employed was essentially the same as that used by Thurman et al. (1951). The larvae were concentrated in wooden troughs and the P^{32} introduced into the water as an H_3PO_4 solution. The P^{32} was supplied by Tracerlab, Inc. on allocation from the Isotopes Division, U. S. Atomic Energy Commission. The dosage of isotope in all four tanks was .019 μc per larva. However, since the larval density varied among the tanks from 3.6 to 5.4 per ml, the rearing solution varied from .066 to .101 μc per ml.

As soon as pupae appeared, the 3′ x 6′ tanks were covered with 3′ x 6′ x 1½′ screened cages in order to retain the adults for simultaneous release. The isotope was introduced in the afternoon of August 16, and the main emergence took place half an hour before sunset on August 19.

A minor release was made on August 18; there were certainly some escapes before then, and a small number left the tanks after the 19th. Although the numbers leaving the tanks within 24 hours of the big simultaneous release of August 19 are uncertain, there is yet no doubt about the overwhelming numbers having been released at the given hour. Attempts to compute per cent emergence failed; there was a very substantial mortality, larval and pupal, in every tank. The release can be given only as approximately one million adults, at least 75% of which left the site half an hour before sunset on August 19.

Four methods were employed for recapturing the marked (radioactive) adults. (1) Thirty-nine New Jersey light traps were operated nightly from five days previous to thirty days after the release; their distribution is indicated in Fig. 1. (2) Two trucks equipped with cone-traps on fender and over cabin roof were operated over set routes three round trips per night for five nights following the release. (3) Aspirator (biting) collections were made at 39 stations, three times at night and three times in the daytime, for six days following the release. (4) Sweep-net collections were made at the same 39 stations on the same schedule. Standard and rigid

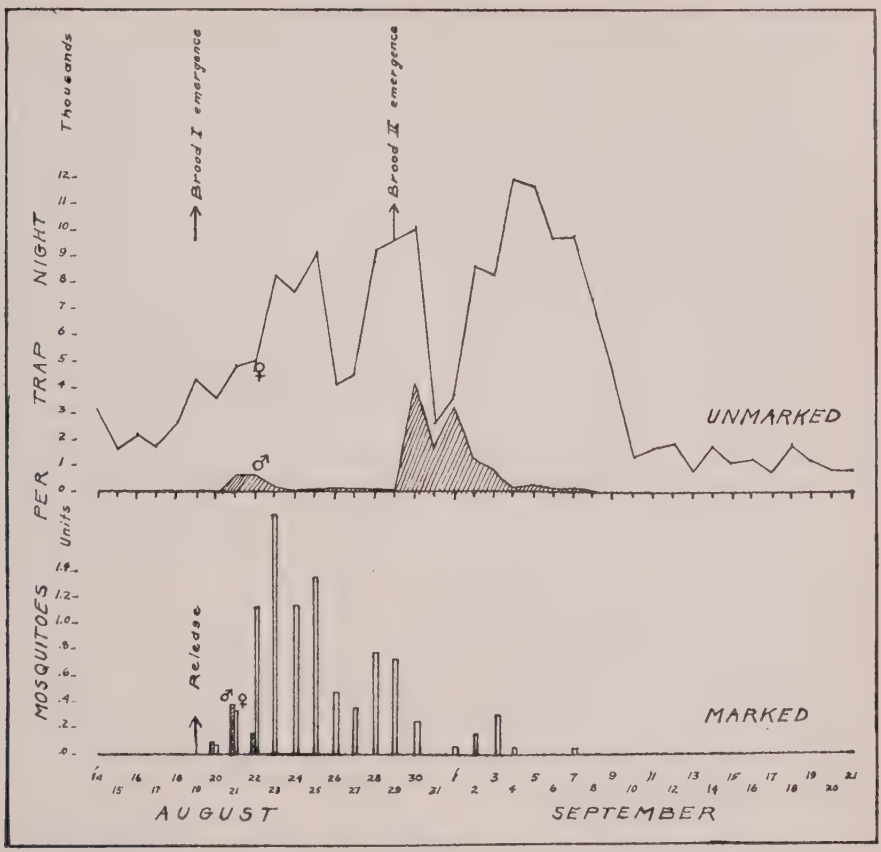

FIG. 2—The relation of the marked brood of *Aedes taeniorhynchus* to the unmarked population. Both graphs based on averages of 33 light traps in the San Carlos Bay area.

procedures and schedules were maintained for each type of collecting. The light traps were concentrated within five miles of the release point, but extended out to 20 miles. The truck trap routes extended four miles from the release point and were each divided into 10 linear portions. The sweep-net and aspirator stations extended out 7 miles from the release point in as uniform a pattern as the terrain and its accessibility permitted. Because of the operating limitations of each type of collection, it was impossible to establish a strict geometrical distribution of recapture points.

All collections were monitored with a laboratory type Geiger-Mueller tube and counter. This was done as quickly as the laboratory force on Sanibel Island could handle the collections. Marked mosquitoes were metered (in counts per minute), sexed, and counted at once. The negative (non-radioactive) portions of collections were set aside for identification, sexing, and counting as time allowed. This latter chore was not completed until February, 1952.

The 10 days of intensive work on Sanibel employed 20 people full time and 6 part time. The light trap work employed two of these an additional three weeks, and

the laboratory work kept three persons occupied some six months.

RECOVERIES OF MARKED MOSQUITOES

1. Light Traps

Radioactive mosquitoes were recovered largely from light traps. Although one of the four traps at Fort Myers Beach did yield one marked female, on August 28, the total collections from these traps, from the Fort Myers trap, and from the trap at Bonita Springs were all extremely small when compared to the other traps used in the experiment. These six traps were in areas of adulticiding control, but whether or not this explained the small collections, it is felt best to restrict our analyses to those traps which all fell within the same performance pattern and where there was no possibility of control interference. The 33 remaining traps, during their 39-day runs (1220 collections) collected 5,986,014 female and 483,661 male *Aedes taeniorhynchus,* for a per-night, per-trap average of 4907 females and 396 males. For the period during which marked mosquitoes entered the traps (Aug. 20 to Sept. 7), 4,610,289 females and 473,344 males were collected, for a per-night, per-trap average of 7546 females and 775 males. In all, 288 marked females and 19 marked males were captured.

A. Females

Distance.—Marked females were caught at the farthest trap operated, 20 miles from the release point (Table II). The largest returns were at traps 9 to 11 miles away on Captiva and Pine Islands. Unfortunately, there were few traps beyond ten miles. Within ten miles of the release point, there was a decrease in number with distance in a northeasterly direction, but an increase in north and northwesterly directions (Fig. 3). The 5 traps within one mile of the release point differed from the two traps 16 and 20 miles away on Pine Island not so much in size of marked-female collections as in their continuity where the distant traps were discontinuous in their returns. All these facts suggest that marked females may have gone, in substantial numbers, beyond the 20 mile limit set by traps.

Direction.—Except for the eastern half of Sanibel Island, where power lines permitted a scattered distribution, the light traps were arranged primarily along three radii from the release point. The northwestern radius followed the outer islands to the northern tip of Captiva. The northern radius crossed Tarpon Bay and up the whole length of Pine Island to Bokeelia at its northern tip. The northeastern radius went to the eastern tip of Sanibel, then across San Carlos Bay, up the road from Punta Rassa on the mainland to Fort Myers. The traps along the northwestern and northern radii caught more marked females than those in a northeasterly direction (Fig. 3). It is particularly noteworthy that the nearest trap on the mainland (6 miles northeast of the release point) is the only one of the 33 traps which caught no marked mosquitoes at all.

Time.—Marked females were caught in light traps from the 1st to the 19th days following the release. The decrease in numbers with time was not constant (Fig. 4). The time distribution showed a very marked cycle with peak catches of 58, 27, 10, and 1, on Aug. 23 (4 days after main emergence), Aug. 28 (5 days later), Sept. 3 (6 days later), and Sept. 7 (4 days later). The same periodicity showed up when the various light traps were grouped according to either distance or direction. This remarkable phenomenon is discussed later.

B. Males

One marked male was caught in a trap two miles northwest of the release point; the remaining 18 were caught in the five traps between ¼ and 1 mile from the release point (Table III). Within such a short distance, direction was irrelevant. In point of time, 3, 12, and 4 respectively were caught on Aug. 20, 21, and 22, or 1, 2, and 3 days after the main emergence.

2. Truck Traps

During their 6 days of operation, the truck traps collected 33,259 female and

Table II. Light trap recaptures of marked female *Aedes taeniorhynchus* in first dispersal experiment. In the first column the letter designations are: S, Sanibel Island; C, Captiva Island; P, Pine Island; M, Mainland. X indicates trap did not run.

Trap Number	From Release Point Miles	Direction	Marked females caught in August-September: 20	21	22	23	24	25	26	27	28	29	30	31	1	2	3	4	5	6	7	Total
S 13	.3	N	1	1	1	5		1	2		1		1									13
S 10	.6	S		2	2		2					2		x								8
S 9	.8	SE									1											1
S 11	.8	SW	1		1	1		2		2	1											8
S 12	1.0	SW		1			2	1	1				1									6
S 8	1.6	ENE			2																	2
S 14	2.1	WNW		1	1	1					2											5
S 7	2.2	E					1	2									2					5
S 6	2.6	ENE		1	3	1		2			3	x										10
S 5	2.7	ENE			2	2			x	3		2							x			9
S 4	2.7	NNE		1	1	4	2	2			6	3	1								1	21
S 3	3.0	NE			2											1						3
S 15	3.1	WNW				1	1															2
S 2	3.9	ENE				1	2			x			1									4
S 16	4.1	WNW					1				1	1										3
S 1	4.3	ENE			2	1	1				4						1					9
P 1	4.4	N			1	1		1				1										4
S 17	5.1	NW		1	7	4	2	4	2	2	1	2						1				26
M 9	6.0	NE																				0
S 18	6.1	NW		1	x	2	3	3														9
S 19	6.9	NW				6	3	3	4	1	1	1				1						20
M 8	7.0	NE				3			x	1		2	2				1					9
C 20	7.6	NW					1	1			1											3
M 7	8.0	NE										1										1
C 21	8.6	NW				2		1														3
C 22	8.9	NW				8	5	3	3								1					20
M 6	9.1	NE					1															1
P 2	9.7	N		1	7	8	3	8	1			4										32
C 23	10.0	NW			4	2	7	4	2		3		1			1	5			x	x	29
M 5	10.4	NE			1							3										4
C 24	11.0	NW				2		4		1		1		x	x	1						9
P 3	16.0	NNW				2		1	x													3
P 4	19.8	NNW					1	1		x	1				1							4
	Total (33 traps)		2	10	37	57	38	44	15	10	26	23	7	0	1	4	10	1	0	0	1	286

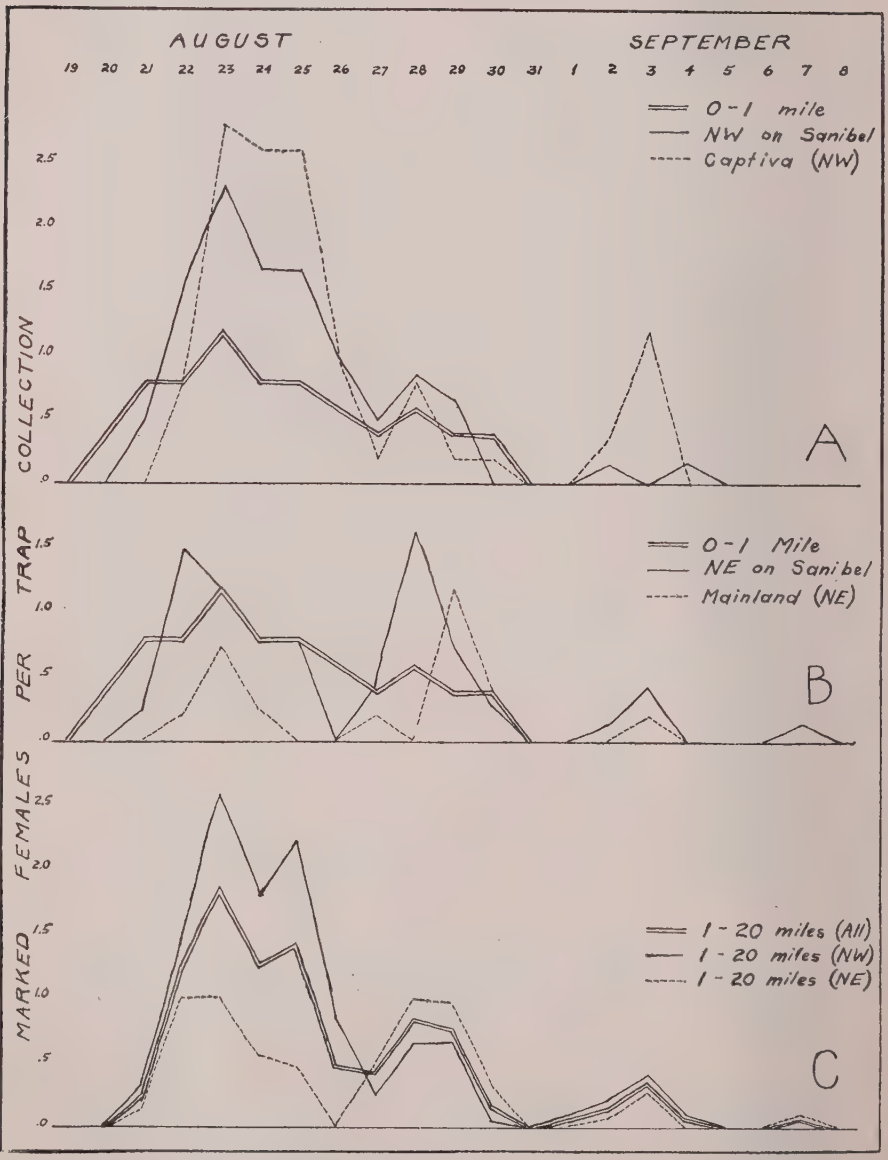

FIG. 3—Comparisons of light attraction periods of marked females as reflected in light trap recaptures.

8,432 male *Aedes taeniorhynchus* in 735 collections. Of this number, 8 females and 1 male were marked (Table IV). The single male capture was at a point on the Gulf beach ¾ miles south of the release point at 0400 on Aug. 24 or five days after the big emergence. Five of the 8 females were caught during the night of Aug. 20–21, three within ½ mile either north or south of the release point and two somewhere between ½ and 2 miles distant, one northwest and the other northeast. One female was caught at 2300 on Aug. 21 within ½ mile of the release point (north or south), and two were caught at 2300 of Aug. 22 somewhere

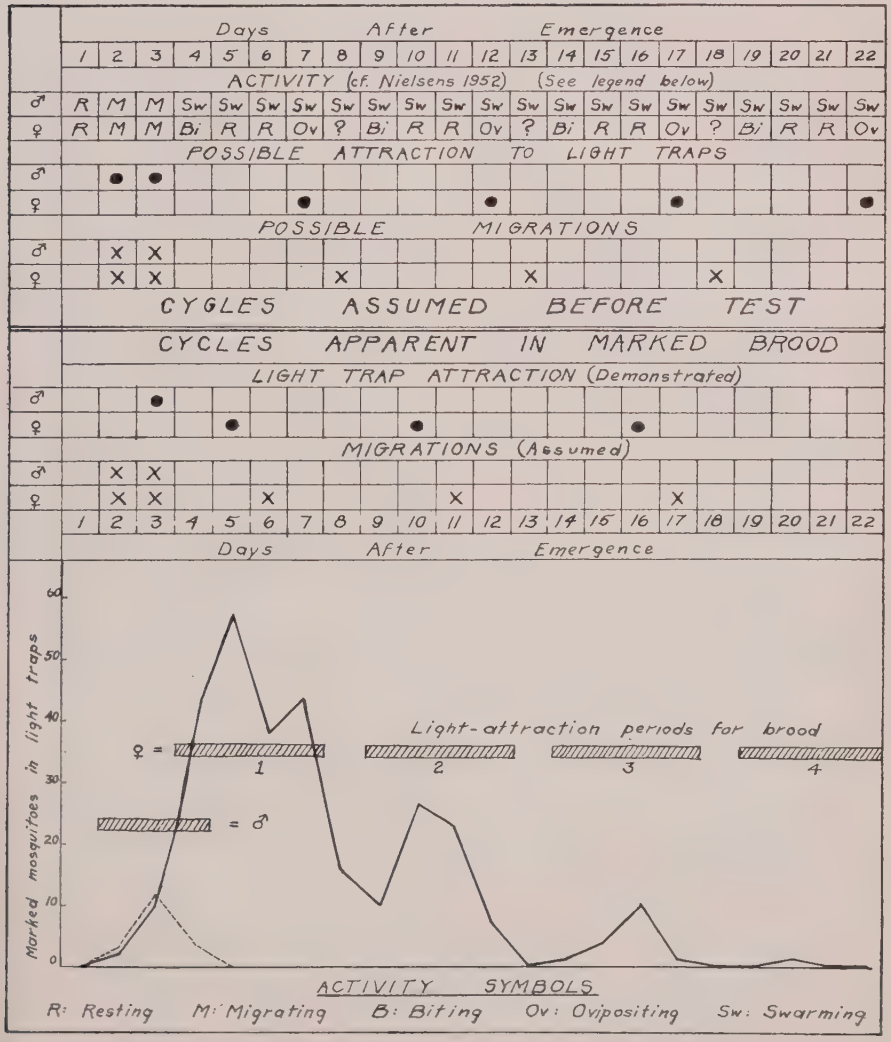
Days After Emergence
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22
ACTIVITY (cf. Nielsens 1952) (See legend below)
♂ R M M Sw Sw Sw Sw Sw Sw Sw Sw Sw Sw Sw Sw Sw Sw Sw Sw Sw Sw
♀ R M M Bi R R Ov ? Bi R R Ov ? Bi R R Ov ? Bi R R Ov
POSSIBLE ATTRACTION TO LIGHT TRAPS
POSSIBLE MIGRATIONS
CYCLES ASSUMED BEFORE TEST
CYCLES APPARENT IN MARKED BROOD
LIGHT TRAP ATTRACTION (Demonstrated)
MIGRATIONS (Assumed)
Days After Emergence
Marked mosquitoes in light traps
0 10 20 30 40 50 60
♀ =
= ♂
Light-attraction periods for brood
1 2 3 4
ACTIVITY SYMBOLS
R: Resting M: Migrating B: Biting Ov: Ovipositing Sw: Swarming

FIG. 4.—Activity cycles of *Aedes taeniorhynchus*, as hypothesized by the Nielsens (1952) and as revealed by the dispersal experiment.

TABLE III. Light trap recaptures of marked male *Aedes taeniorhynchus* in first dispersal experiment. All were on Sanibel Island.

Trap Number	From Release Point		Date in August			Total
	Miles	Direction	20	21	22	
S 13	.3	N	1	1		2
S 10	.6	S		3	1	4
S 9	.8	SE		1		1
S 11	.8	SW	1	6	2	9
S 12	1.0	SW	1	1		2
S 14	2.1	WNW			1	1
		Total	3	12	4	19

between 2 and 4 miles northeast of the release point.

3. Sweep-Net Collections

The 39 sweep net stations during their week of operation yielded 9,651 female and 426 male *Aedes taeniorhynchus* in 1033 collections. The only marked mosquitoes caught were two females at 1011 on Aug. 21, ¾ miles southwest of the release point and one female at 0847 on Aug. 19, one mile west of the release point. It is noteworthy that this latter mosquito was caught 10 hours before the main release.

4. Aspirator Collections

The same 39 stations yielded 1049 collections to the aspirator for a total of 13,906 females and 37 males, the latter accidentally having rested on the bodies of the collectors. The only marked mosquito thus caught was a female at 0810 on Aug. 23 at a station 7 miles northwest of the release point.

5. Miscellaneous Collections

A few male swarms were collected by sweep net, but the relatively small samples yielded no marked mosquitoes. Five light traps were experimentally operated without lights, paired with the standard traps within a mile of the release point. The "blind" trap ¼ mile northeast of the release point caught one marked female on Aug. 22, and the "blind" trap one mile southwest caught a marked female on Aug. 23. The laboratory and headquarters for the experiment were at the lighthouse station at the eastern tip of Sanibel Island. Mosquitoes gathered in considerable numbers on the white building and a few sweeps under the high eaves with a sweep net (on the end of a bamboo pole) between 0115 and 0200 on Aug. 24 yielded 16 male and 13,801 female *Aedes taeniorhynchus*. Two females were radioactive. This was 4¼ miles east of the release point.

TABLE IV. Truck trap recaptures of marked *Aedes taeniorhynchus* in first dispersal experiment.

Time		From Release Point		Sex
Date	Hour	Miles	Direction	
Aug. 20	2300	0.0–0.5	N or S	Female
"	"	"	"	"
"	"	"	"	"
Aug. 21	0400	0.5–2.0	NE	"
"	"	"	NW	"
"	2300	0.0–0.5	N or S	"
Aug. 22	"	2.0–4.0	NE	"
"	"	"	"	"
Aug. 24	0400	0.6–0.8	S	Male

Discussion

Dispersion and migration in insects have been so diversely interpreted that the two terms mean little without definition. In the mosquito field, this is traceable to the universal prompting to consider migration merely a matter of long distances being covered. The only hope of bringing order to our terminology lies in examining the objective causality, or ethology, of flights resulting in dispersion,—dispersion meaning according to the dictionary, simply "distribution from a fixed source." Dispersion results whenever a member of an insect population takes wing. While resting, that insect may be in any of several physiological states and this state will determine its reflex response to any external stimulus. Those physiological states characterized by a neural unbalance or unsatisfaction (e.g. hunger, thirst, rut) are accompanied by an appetence: an active seeking of the external stimuli associated with the satisfaction of the physiological want. Flight impelled by appetence, we may call *appetential* flight. It receives internal direction, whatever may be the influence of external stimuli. The insect, nevertheless, cannot be constantly under the nervous tension of a physiological need, so that there must be a physiological state of satiety which from the standpoint of reflex possibilities for that insect would be strictly a neutral state. While in this state, the insect is still sentient, of course. Since, however, there is no appetence, responses (if any) to external stimuli will be purely mechanical and with external direction only. Should any external stimulus cause an insect in a neutral state of rest to fly, the result will be a *non-appetential* flight. Such flight is without internally influenced orientation. We believe that insect migrations are examples of non-appetential flight, a special flight which serves no special physiological need, whatever teleological end it may subserve.

Tinbergen (1951) in his excellent synthesis of innate behavior appears to infer that the flow of motivational impulses through the nervous system is continuous so that an animal at all times must be either behaving "appetitively" * or consummating the act resulting from such behavior. "In extreme cases the appetitive behavior may be prolonged and highly adaptable, as in the migratory behavior of animals" (p. 105). Since appetential behavior is admittedly purposive, Tinbergen is in opposition, at least as far as insects are concerned, to Uvarov (1928), Kennedy (1951), and others who insist that insect migrations are not directed to a goal.

Appetential flight (e.g. search for food, shelter, mates, oviposition sites, etc.) will necessarily result in a certain amount of dispersion. This type of dispersion is common to all mosquito species since it reflects flight activity incidental to the satisfaction of the biological needs of a lifetime. A multitude of appetential flights enters into the ultimate dispersion of a population originating at one point. So diversified and numerous, indeed, are these flights that the resulting "normal" dispersion will be random, omnidirectional, and with limits a function of (1) density at the source, (2) longevity, (3) average length of component flights. The pattern of this normal (for want of a better word) dispersion may be affected by the distribution of shelter, food, etc. and possibly also by weather, but on the whole it tends to be radial with the distribution along any radius typically a hyperbolic regression. It must be noted, however, that there are long appetential flights of a special nature which should not be confused with true migration; a classic example would be the hibernational flights of many anopheline species.

In such a species as *Aedes taeniorhynchus,* migrational flight effects a dispersion which must be superimposed on the normal dispersion resulting from appetential flights. The ultimate distribution or dispersion of a population of migratory mosquitoes may be determined

* According to dictionary, "appetitive" and "appetitively" are derivatives of *appetite.* Since we are concerned here with *appetence* (also in dictionary), the derivatives thereof should be "appetential" and "appetentially."

more by a single flight participated in by all individuals than by the subsequent multiplicity of appetential flights performed individually. Such factors as density at the source and longevity, which have much to do with setting the limits of normal dispersion, probably have very little effect on migratory dispersal limits. Conversely, weather and topography may exert the major influence on orienting or channeling migratory flight, and hence may be the most important determinants of the dispersion pattern for a population of migratory mosquito species. It follows, therefore, that for non-migratory mosquitoes the pattern and limits of dispersion will be set largely by biological needs of the insects, whereas for migratory mosquitoes they will be determined largely by meteorological and topographical accident.

Assuming that "flight for flight's sake" is the cause of a special migratory dispersion in some mosquito species demands some sort of explanation. It is suggested that both males and females of *Aedes taeniorhynchus* respond to an undefined light factor at dusk and dawn (for discussion with respect to male swarming, cf. Nielsen and Greve, 1950) by ascending from their resting places and flying. These are the primary, non-appetential responses, common to both sexes, in reaction to the same stimulus. Physiologically, this flying is non-motivated and non-purposive. Once on the wing, with no internally orienting influences of the appetential type, the direction of their flight will be determined by purely external factors. Migration then ensues as a flight in which the orientation of the insects' bodies, specifically, is non-purposive and at the mercy of external stimuli.

This secondary effect, migration, may be modified or arrested but the primary response, flight, is presumed to occur every dawn and dusk as a robot phenomenon. Newly-emerged adults may delay their primary response beyond dusk if they have not by that time attained an age at which sustained flight is possible; i.e., the exodus may occur at any hour of the night. In females the secondary effect may be arrested by heaviness of abdomen as a result of a blood meal or gravidity. Since these circumstances endure perhaps three consecutive days, migrations beyond the initial exodus from the emergence site would occur on a five-day cycle, i.e. during the permissive interval between oviposition and the next blood-meal (Fig. 4). In the case of males, it is necessary to consider the possible intervention of appetential behavior. It is conceivable that males accompany females in the initial exodus merely because the primary response is at once diverted into a mating pursuit of the females. This would be the mass counterpart of individual males dropping out of swarms, later in life, to copulate with females flying nearby.* Since later female migrations are not mass phenomena exerting a mass stimulus, the males perform no further migrations but swarm every twilight the rest of their lives, as observed by the Nielsens (1952), with copulation accidental only since nearly all females have been impregnated during the initial exodus. It is furthermore conceivable that the swarming of males as well as the strange oviposition flights of females described by the Nielsens (1952) may be diversions of the secondary effect consequent upon fulfillment of the same primary response hypothecated for both sexes.

The findings of any dispersion experiment are completely at the mercy of the sampling techniques used, both in collecting mosquitoes for marking and in collecting them later for re-identification. In the case of *Aedes taeniorhynchus,* for instance, marking and releasing mosquitoes caught by light trap or bait trap would yield a most untrue picture of dispersion from breeding areas if it happened, as appears to be the case, that the main migratory flight occurs before the animals start the blood search and before they can react positively to light. This bias was avoided

* Mating in swarms was first observed for *Aedes taeniorhynchus* by Mr. James S. Haeger on Sanibel Island, Florida, December 3, 1951. It never occurred in the swarms which the Nielsens observed so closely in the Fort Pierce area in 1951.

in this experiment by marking the insects in the aquatic stages, thus enabling a tracing of their dispersion back to a true and natural origin in both time and space. This elimination of bias is not achievable in sampling the population for recaptures. For *Aedes taeniorhynchus,* and certainly for many other mosquitoes, there is no collecting technique known which will sample the entire population. All methods are selective: light traps sample that segment of the population which is on the wing and responding positively to light, bait traps or biting collections sample only that population segment (all female) which is at the time searching for blood, nets of any kind sample only those segments of the population which are either on the wing at the time or resting in the type of vegetation swept at the time, and so on. Interpretation of recaptures in a marking-releasing-recapturing experiment must therefore conform to the techniques employed in recapturing. Although we tried in this experiment to employ a variety of recapturing techniques in order to circumvent sampling selectivity, it developed that only the light traps yielded significant numbers of recaptures. Interpretation of our findings is consequently inseparable from an understanding of the light trap reactions of *Aedes taeniorhynchus.*

1. Females

The most significant finding of this experiment was the periodicity of marked female numbers in the light traps. On the basis of phototaxis studies in the laboratory and visual observations in the field, the Nielsens (1952) have advanced the working theory that in *Aedes taeniorhynchus* the females react positively to light approximately on their seventh day of adult life and cyclically about every fifth day thereafter. Except for the initial light attraction being two days earlier, our dispersal study contributes striking confirmation of this theory (Fig. 4). From the standpoint of light trap analysis, it is now fairly certain that female numbers must hereafter be interpreted with this phenomenon in mind. Studies in progress will clarify the rigidity of the gonotrophic cycle, but it is obvious even now that for a brood of *taeniorhynchus,* the overlap of individual light attraction cycles will result in a *light trap number* x *time* distribution with a wave pattern reflecting the degree of isochronism in the emergence.

Interpretation of light-trap data on female *taeniorhynchus* is further complicated by the migration periods. The Nielsens (1952) give convincing evidence that "dispersal" (= migration, in our sense) occurs sometime between the first and fourth days of adult life,—previous to the onset of biting activities and to the first light attraction period. They found biting activities to be concentrated on a five-day cycle,—4th, 9th, 14th, etc. days after emergence, while ovipositing followed the same cycle three days later,—7th, 12th, etc. days after emergence. They do not, however, discuss the possibility of migratory flights occurring also every five days, say, on the 2nd, 8th, 13th, etc. days of adult life. In the experimental results under discussion, the fact that traps in a northeasterly direction collected as many marked females in the second light attraction period as in the first, while traps to the northwestward collected far more in the first than in the second (Fig. 3-C) suggests that a migration may have taken place in the early days of each gonotrophic period. The odd pattern on Captiva Island (Fig. 3-A) with the third period bringing in more marked mosquitoes than the second, although a full six days of progressive mortality intervened, seems also to indicate brood shifts at each period. The same applies to the pattern at Sanibel trap #4 (2.7 miles NNE) where the second period brought in more marked females than the first, the third none at all, and the fourth one single mosquito. If there were no further migrations after the initial one, the basic dispersal (non-appetential) would be ended before the first light attraction period and the distribution essentially the same for all light attraction periods. Consequently, the pattern shown at the bottom of Fig. 4 for all

recaptures, with each successive modulation smaller than the previous one in accord with mortality rate, would prevail at each trap location or group of locations. This definitely was not the case.

The two concepts of female *taeniorhynchus* migration that have been discussed, periodicity and non-purposiveness, lead to an entirely different dispersal pattern from any discussed in the voluminous literature on mosquito dispersal, although results obtained by Clarke (1937, 1943) and Horsfall (1942) with other migratory species (*Aedes vexans* and *Psorophora confinnis* respectively) resemble ours on several points. It is a pattern of random dispersal (appetential) periodically repeated from foci established by the preceding migratory dispersal (non-appetential), —all very likely conditioned by meteorological and topographical exigency. Since the latter circumstances may vary at each migration, the net dispersal for a brood is not only very complex but infinite in its possibilities. The one dispersal pattern rendered impossible is the purely radial type with densities a simple function of distance from point of origin.

With such a dispersal pattern, the area of occupancy by a brood from a single origin would, in the absence of weather or terrain influence, expand cyclically. The density of mosquitoes at each expanding perimeter would be decreased according to a calculable regression which would be a function of the longevity factor and area factor. If the entire brood were to align itself evenly along the ten mile perimeter after the first migration, the second 10-mile migration would bring 3.2% of the original brood back to within a mile of the point of origin. This figure, of course, assumes no mortality, no weather deflection, and 100% migration. The return to origin would result in approximately the same density within a mile of the origin during the second light attraction (= oviposition) period as prevailed within a mile of the 10-mile perimeter during the first oviposition period. What is more likely than an alignment on any perimeter, however, is a radial distribution on a normal (probability) curve with a peak, say, at the 10-mile perimeter. Since area on any perimeter is inversely proportional to the radius, the actual distribution of mosquito densities would be shifted somewhat to the origin side of the maximum. Thus, if the mean distance of a migration were 10 miles, the density on the 5-mile perimeter would be 2/3 that on the 10-mile perimeter (probability curve factor x area factor, 1/3 x 2) while on the 15-mile perimeter the density would be 2/9 that on the 10-mile perimeter (1/3 x 2/3).

For the first light attraction period of the marked brood, the light trap recoveries appear to fit roughly into such a curve. It is clear, however, that any shift in dispersal resulting from meteorological or topographical influences could alter these density proportions. The data indicate a northwestward shift of this nature, which could well account for the presence of marked females close to the release point,—for theoretically none should linger here during the first light attraction period.

At this point it would be well to emphasize that random, omnidirectional dispersion of the type hypothesized above has not been demonstrated for *Aedes taeniorhynchus,* even as a primary trend subject to later deflections by weather or other factors. Field observations on several occasions have shown rather a unidirectional exodus, and it is highly probable that winds and topography exert a channeling effect on the migration. Although the directional data from the present experiment are definitely prejudiced by the distribution of light traps, the fact remains that the traps in a northeasterly direction yielded only a fraction of the returns produced in the traps to the north and northwest at any distance, on or off Sanibel Island (Fig. 3). This northwestward deflection may have resulted from either a meteorological influence, the prevailing southeasterly winds (Table I), or a topographical influence, the alignment of the coast and islands, or a combination of both.

The fact that *Aedes taeniorhynchus* rarely occurs in nuisance proportions more than five miles inland from tidewater (Provost, 1951) in spite of its now demonstrated much longer flight range is itself evidence that migrations must largely parallel the coastline. This fact in conjunction with the repetition of migrations by the same brood could well result in oviposition (in second or later periods) at the site of origin in spite of the remarkable exodus soon after emergence.

2. Males

It is usual with light trap catches of *Aedes taeniorhynchus* for females to outnumber males by a considerable margin. It is absolutely essential that the reasons for this be understood before any male light trap data are interpreted. We must digress here to prove our points: (1) positive phototaxis is as strong in males as in females, (2) female collections average larger than male collections because the larger ambit of appetential flight activity, in place and in time, brings more of them within the orbit of light trap attraction, and (3) large male collections are purely a matter of chance concentrations at the trap site.

The history of *Aedes taeniorhynchus* light trap captures in Florida reveals that females exceeded 100,000 in a night's collection once (265,000) and males did it twice (117,000 and 125,000). Obviously there are circumstances under which males will come to traps in as large numbers as females do. In the course of the experiment, the five light traps within a mile of the release point were paired with "blind" traps, i.e. regular light traps run without a light; the two traps were 20 feet apart at each location. The blind traps caught only a fraction of what the lighted traps caught. It was found (Table V) that the preponderance of females over males was almost invariably greater in the unlighted than in the lighted traps. During the peak days following the two big emergences of the experiment interval, the female to male ratio averaged 2.4 times as great in the unlighted as in the lighted traps. This would certainly indicate that, *at the trap site,* males were attracted to light as much or very likely more than females. We have on record hundreds of collections in which males far outnumbered females.

An analysis of large collections of either sex from the San Carlos Bay area (Table VI), 1946 to the present experiment, reveals that as the size-of-collection category increases, the ratio of female to male collections within these categories decreases. Collections of over 50,000 are about equally divided between the sexes, but as the size diminishes the ratio of female to male collections increases until it reaches approximately 10:1 for collections between 5,000 and 20,000. It is plain, therefore, that more females than males are caught in light traps not because female collections are larger but because large collections of females are more frequent. The sedentary nature of post-migration males compared with females would result in the traps sampling much larger segments

Table V. Sex ratios of *Aedes taeniorhynchus* caught in New Jersey traps with and without light at five locations on Sanibel Island, August 21–26, and Aug. 30–Sept. 7, 1951.

Trap number	Average per trap night				Female/male		Light attraction male>female
	trap with light		trap without light		light	no light	
	female	male	female	male	1	2	2/1
9	3,709	1,297	78	15	2.86	5.20	1.82
10	5,954	779	206	5	7.64	41.20	5.39
11	7,580	2,193	389	42	3.45	9.26	2.68
12	2,887	889	178	42	3.25	4.23	1.30
13	14,173	1,544	764	26	9.17	29.38	3.20
Average	6,928	1,354	324	26	5.11	12.46	2.44

TABLE VI. Analysis of *Aedes taeniorhynchus* light trap catches exceeding 5000 in either sex, San Carlos Bay area, Florida. A = semi-weekly catches, 1946 to Aug. 14, 1951. B = daily catches Aug. 14 to Sept. 21, 1951. C = daily catches during first light-attraction period of Brood II, Aug. 29 to Sept. 4, 1951. D = daily catches during first light-attraction period of Brood I, Aug. 20–26, 1951. The marked mosquitoes of the dispersal experiment belonged to Brood I (column D).

Size of catch in thousands	Number of Collections								Ratio of female collections to male catches in each size			
	Females				Males							
	A	B	C	D	A	B	C	D	A	B	C	D
5–10	103	218	60	52	10	9	8	1	10.3	24.2	7.5	52.0
10–15	52	91	29	28	5	4	3		10.4	22.8	9.7	—
15–20	13	48	18	11	1	2	2		13.1	24.0	9.0	—
20–25	8	16	10	3		1	1		—	16.0	10.0	—
25–30	9	14	3	4	2	2	2		4.5	7.0	1.5	—
30–35	5	2	1		1				5.1	—	—	
35–40	3	2	2						—	—	—	
40–45	5								—			
45–50	2	1	1		1				2.0	—	—	
50–55	1	2			1				1.0	—		
55–60												
60–65	1								—			
65–70	2				1				2.0			
70–75	1								—			
75–80												
80–85	1				1				1.0			
85–90		1								—		
90–95												
95–100					1				*			
100 +	1				2				2.0**			
Total	207	395	124	92	26	18	16	1	8.0	22.0	7.8	98.0

— = Only female collections.
* = Only male collection.
** = Reversed ratio, i.e. male to female.

of the female population than of the male, and this is a more plausible explanation of female preponderance in traps than the assumption that light attraction *per se* is stronger in females.

Through most of their post-migration lives, males are so highly sedentary (cf. Nielsens, 1952) that large collections such as are given in Table VI may be purely accidental, i.e. there just happened to be large concentrations of migrating males at the immediate site of the trap. Some evidence for this is found in the fact that these large male collections are more localized than large female collections; for instance, of the 26 collections in the first male column of Table VI, 22 were at one trap and this included all those of over 20,000, and of the 18 large male collections made during the dispersal experiment, 12 were at 5 of the 33 traps.

It is possible therefore to explain the small number of male recaptures in this experiment on the assumption that the males of this brood just did not happen to be concentrated at the trap sites on August 20–22. This assumed behavior among the marked males is corroborated among the unmarked males of the same brood. The males of Brood I (emerging Aug. 18–19) simply did not enter traps as did those of Brood II (emerging Aug. 29–31). The difference (Table VI), however impressive for the entire trapping record of the experiment, was nevertheless attributable to but a few adjoining traps

(chiefly Sanibel Nos. 14, 15, 16, 17) and can therefore be explained as a matter of chance concentrations of migrating males at trap sites.

The limited light trap recaptures (19 vs. 288 females) need not be attributed to a biased sex ratio at release. Estimates at that time, if anything, favored males. Both the short duration (3 days) of marked male recapturing and the short distance (2 miles) involved must be interpreted in the light of factors discussed above.

On the point of longevity, the Nielsens (1952) have demonstrated that the swarming period of males extends from "the age of 4 days until they are 2–3 weeks old." The disappearance of males from light trap collections at the age of 4 days in this experiment suggests that (1) they are not attracted to light during the swarming period of their lives, or (2) they are so sedentary during this period as not to even approach the vicinity of traps. The observations of the Nielsens substantiate both of these possibilities. Since the light trap history of *taeniorhynchus* abounds with a sprinkling of male captures long after main emergences, it is extremely difficult to believe that males are never attracted to light beyond their fourth day of adult life. It is more likely that between swarming intervals, i.e. in the middle of the night, they may be positively phototactic but seldom occur in traps because of their extreme sedentariness at this age.

It appears, therefore, that the main period of male attraction to light traps coincides with the "disappearance" from the breeding marshes emphasized by the Nielsens and interpreted by them as the period of dispersal. During this same dispersal period, Aug. 20–22 in this experiment, the females reached a distance of 20 miles, —although their light reactions did not permit them to enter traps in force until after the males no longer did so. Why then were not many more males caught at distant traps on Aug. 20–22?

If the males did not disperse beyond two miles, there should have been many more trapped within this distance. If the sex-ratio at release was 50–50, considerations of mortality and area would have resulted in a far greater density of males within one mile of the release point on Aug. 20–22 than of females within twenty miles on Aug. 22–25. Yet the per-trap, per-night captures of the latter (1.33) actually exceeded the former (1.20).. Two possible explanations of this phenomenon may be considered. (1) The males accompany the females in their full migratory dispersion but since females are not attracted to light at this age, the males are likewise kept away from the orbits of light trap attractions; the male recaptures of this experiment would then represent non-migrants. (2) The males accompany the females in the initial exodus a short distance only and then fall out of migration; the small number of male captures within their small dispersion area would then indicate a lesser attraction to traps than that of females. The demonstration above of a lesser attraction to light traps for males of this brood makes the latter, favored explanation possible, but it does not disqualify the former.

Summary

1. Radioactive *Aedes taeniorhynchus* adults were produced by exposing the larvae to radiophosphorus (P^{32}). The subsequent dispersion from Sanibel Island, Florida, was studied from the time and space distribution of marked recaptures. Other collecting methods were used but only light traps yielded significant numbers of recoveries.

2. Migration, as a special non-purposive flight, occurs from 1 to 4 days after emergence. This initial exodus carried females to the limits of the collecting area, 20 miles, and probably beyond. The males probably did not migrate beyond 2 miles. This main flight was deflected to the northwestward, possibly by prevailing southeasterly winds and by the NW-SE alignment of the coastline and topography.

3. Light traps collected females on a 5-day cycle of numbers; the last one was caught 19 days after emergence. Males entered light traps the first three days only after emergence; this has no bearing on their longevity.

4. There is some indication that females may migrate early in each 5-day progenitive period.

5. Dispersion of female *Aedes taeniorhynchus* appears to be a random, omnidirectional, appetential dispersal periodically repeated from foci established by a previous migratory, channeled, non-appetential dispersal. Males accompany the females on the initial exodus but probably drop out of the migration within a mile or two and thereafter settle down to a sedentary life characterized by swarming during twilight periods for 2–3 weeks.

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CHLORIDE ION TOLERANCE OF FOUR MOSQUITOES OF OKINAWA[1]

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While engaged in mosquito survey work on Okinawa during 1948–1950, an investigation was made to determine whether any appreciable salinity tolerances existed among the mosquito larvae of that area. It was felt that sea water, barring the existence of salt or brackish water mosquitoes, might be successfully employed as a control measure in the fire barrels of those installations located near the ocean. This paper describes the larval distribution of *Anopheles hyrcanus sinensis* Wiedemann, *Culex tritaeniorhynchus* Giles, *Culex quinquefasciatus* Say, and *Culex bitaeniorhynchus* Giles with respect to different salinities and tolerances of these species to sodium chloride solutions.

Method. Unless unusually heavy breeding was encountered, a standard of 50 dips in most cases was taken in each collection. Specimens collected were placed in 95 per cent alcohol and later examined microscopically for identification. Keys in Bohart and Ingram (1946) were used for making determinations. Total chloride analyses of the water samples were made by Mohr's method of titration with silver nitrate, the determinations being made within forty-eight hours. To facilitate comparison, soluble chloride values of samples tested are expressed as per cents of the soluble chloride content of sea water. Sea water adjacent to Okinawa was determined to contain 19.72 grams of total chlorides per 1,000 grams of sample.

Characteristics of Areas Sampled. Areas chosen for salinity observations were mainly low, extensive marshes and tidal mud flats located adjacent to the ocean. The majority of them were separated from the sea by walls or dikes. However, faulty flood gates and imperfections in sea walls caused by erosion and faulty construction rendered many of them at least partially defective which permitted the inflow of sea water. Streams and canals with outlets to the sea received minor attention since tidal fluctuations were likely to cause wide variations in chloride content within relatively short periods of time.

One area of particular interest which received major attention was a flat, low-lying peninsula of approximately 2,000 acres surrounded by the sea on three sides. This was located on the eastern side in the southern third of the island. It contained several extensive marshes in addition to tidal streams and canals. Apparently a major portion of this area formerly had been utilized in the production of rice, as remnants of paddy banks were much in evidence. This peninsula was separated from the sea by the ruins of a sea wall on one side and a series of sand banks on the remaining two sides through which had been cut ditches. Water poured through these during periods of high tide and collected in numerous depressions, thereby affording a series of water collections of varied chloride concentrations.

Species Distribution in Relation to Salinity. Sampling was begun in September 1949 and was continued until May 1950. A total of 14,976 dips was taken in the 310 areas investigated. The following larvae were collected in the numbers indicated: 2,970 *Culex tritaeniorhynchus,* 1,067 *Culex quinquefasciatus,* 691 *Anopheles hyrcanus sinensis,* and 10 *Culex bitaeniorhynchus.* A summary of these results is listed in Table I. Salinity values of sam-

[1] This paper is based on a thesis presented to the Graduate School of the University of Maryland in partial fulfillment of the requirements for the degree Master of Science. The writer is indebted to Dr. E. N. Cory, State Entomologist of Maryland for advice and encouragement.

TABLE I.—Larval Densities in the Chloride Ranges Sampled

Percent of soluble chloride content of sea water	Number of dips taken	Larvae per dip			
		C. tritaeniorhynchus Giles	*C. quinquefasciatus* Say	*C. bitaeniorhynchus* Giles	*Anopheles hyrcanus sinensis* Wiedemann
100–96	50				
95–91	270				
90–86	100				
85–81	50				
80–76	No samples taken				
75–71	No samples taken				
70–66	140	.04			
65–61	100				
60–56	50				
55–51	150	.01			
50–46	200				
45–41	100				
40–36	160	.04			.008
35–31	250	.04			
30–26	350	.04			.009
25–21	790	.30			.006
20–16	950	.19			.001
15–11	1723	.12	.002		
10.0–9.0	400	.33			
8.9–8.0	750	.13	.001		.001
7.9–7.0	223	.31	.08		.009
6.9–6.0	191	.86			
5.9–5.0	458	.47			.02
4.9–4.0	750	.26	.004		.05
3.9–3.0	1042	.24	.003	.003	.06
2.9–2.0	1915	.34	.004	.002	.11
1.9–1.0	2201	.17	.14	.0005	.10
.99–0	1613	.10	.45	.001	.08

ples are expressed in the percentage of sea water that they contained using total soluble chloride values as a means of comparison. These values are presented in increments of one from 0 to 10 per cent and in increments of five from 11 to 100 per cent. No water in the range of 71 to 80 per cent sea water was sampled. Since no chloride testing was done in the field, the concentration was unknown at the time of larval survey. This also accounts for the small amount of collecting done in water containing more than 35 per cent sea water. Larval occurrence was sporadic in the higher ranges and was not consistently encountered in saline concentrations greater than 40 per cent sea water.

Culex tritaeniorhynchus were found rather consistently in all saline ranges up to those of 40 per cent sea water. One collection of 5 larvae was taken from a tidal stream near its confluence with the ocean in water containing 67 per cent sea water. Two additional larvae were collected in a similar situation, in which the water contained 53 per cent sea water. However, it is felt that the above two collections are of questionable significance as subsequent samples taken from these areas yielded much lower values. This species was not found to approximate its fresh water density of 0.19 larvae per dip in situations greater than 25 per cent sea water. It was taken in the greatest density in areas containing 6.0 to 6.9 per cent sea water, suggesting that possibly this is the optimum range for this species.

Anopheles hyrcanus sinensis was found only in water of saline ranges from 0–35 per cent of sea water, but was only collected in significant numbers in the 0–5.9 per cent sea water range, and in densities

TABLE II.—Larval Mortality Rates in Various Concentrations of Sodium Chloride Solutions

Culex tritaeniorhynchus Giles

Per cent of NaCl	Number Started	Cumulative Mortality at 24 hour Periods in Per cent														
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
distilled water	50	20	26	34	48	52	56									
0.25	40	10	15	25	32	40	42	42	45	45	45	45	47			
0.50	40	15	20	27	35	45	47									
0.75	10	10	30	30	30	40	50	50								
1.00	50	36	50	60	66	68	72	74								
1.25	20	25	80	85	90	95	95									
1.50	20	20	80	100												
1.75	20	70	95	95												
2.00	20	80	100													

Anopheles hyrcanus sinensis Wiedemann

Per cent of NaCl	Number Started	Cumulative Mortality at 24 hour Periods in Per cent														
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
distilled water	30	30	43	37	60	60	67	70	80	87	93	97	97	100		
0.25	20	30	40	50	50	55	60	60	75	75	75	75	75	80	80	80
0.50	20	15	50	50	55	60	75	75	80	80	80	85				
1.00	30	67	90	90	90	90	90	90	100							

Culex bitaeniorhynchus Giles

Per cent of NaCl	Number Started	Cumulative Mortality at 24 hour Periods in Per cent														
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
distilled water	15	48	54	74												
0.25	15	27	54	54	80											
0.50	15	19	60	60	67	67										
1.00	15	40	100													

Culex quinquefasciatus Say

Per cent of NaCl	Number Started	Cumulative Mortality at 24 hour Periods in Per cent														
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
distilled water	10	20	20	20	20	*										
0.25	10	0	10	10	10	*										
0.50	10	10	10	10	20	*										
0.75	10	10	10	40	40	*										
1.00	10	60	90	90												
1.25	10	100														
1.50	10	100														
1.75	10	100														

* Discontinued on the fifth day.

exceeding its two year average of 0.04 specimens per dip in the 0 to 4.9 per cent sea water range. It was taken in the greatest density in the range of 2.0 to 2.9 per cent sea water, suggesting an optimum for this species.

Culex quinquefasciatus was found in numbers that approached its fresh water density of 0.49 only in the 0–0.99 per cent range. Extensive collecting was done on the peninsula previously described where the drainage from a large native village flowed into the area. Normally this material which contained effluent from animal pens and cesspools would have afforded ideal breeding areas for this species. However, none was found in any range higher than the above figure.

Culex bitaeniorhyncus was not taken in densities that approached its two-year

average of 0.04 larvae per dip in any saline range. The scarcity of this species in the situations investigated might be attributed at least partially to its preference for clean water in shaded situations, since the vast majority of the areas sampled were relatively unshaded.

LABORATORY OBSERVATIONS. Limited laboratory tests were conducted at room temperature to determine which of those species had the most tolerance for sodium chloride solutions. Third and fourth stage larvae were placed individually in 10 cc capacity bottles containing various concentrations of sodium chloride as shown in Table II. Checks of distilled water were run in each test and pablum was used as food. Observations were made at twenty-four hour intervals.

High mortality rates were encountered in checks of the three species on which observations were completed. It is felt that had checks of water from rice paddies or other natural habitats of these species been used, mortality rates in checks would possibly have been lower. The average chloride content of water tested from 16 rice paddies was found to be 0.35 per cent of the chloride value of sea water.

In all cases *Culex tritaeniorhynchus* exhibited the lowest mortality rates. Adult emergence in the case of this species occurred in all sodium chloride solutions up to and including 1.25 per cent. Adult emergence did not occur in the cases of *Anopheles hyrcanus sinensis* and *Culex bitaeniorhynchus* in sodium chloride solutions in excess of 0.50 per cent.

SUMMARY AND CONCLUSION. Tests were made at Okinawa during 1949 and 1950 to determine the feasibility of using sea water in fire barrels as an anti-mosquito measure. Chloride ion determinations were made of water taken from 310 areas. *Culex tritaeniorhynchus* larvae were found in water with a chloride content as high as 67 per cent of the chloride value of sea water, but were not collected consistently in water with a chloride value greater than 40 per cent. *Anopheles hyrcanus sinensis* larvae were encountered in chloride ranges as high as 33 per cent, but were taken in significant numbers only in ranges up to 6 per cent of sea water. *Culex quinquefasciatus* larvae were taken in quantity only in water containing up to 4 per cent of sea water chloride content.

Tests were conducted to determine what tolerance these species possessed for sodium chloride solutions. *Culex tritaeniorhynchus* larvae exhibited the highest tolerance. Tests on *Culex quinquefasciatus* larvae were incomplete. Larvae of *Culex bitaeniorhynchus* and *Anopheles hyrcanus sinensis* exhibited approximately equal tolerances.

Culex tritaeniorhynchus larvae exhibited the highest tolerance to soluble chlorides. However, *Anopheles hyrcanus sinensis* larvae were also collected in water of fairly high chloride content. It is felt that because of the dilution factor, due to heavy rainfall, use of sea water to prevent or inhibit the breeding of these two species in fire barrels would be inadvisable. *Culex bitaeniorhynchus* and *Culex quinquefasciatus* would probably be prevented from breeding for prolonged periods of time.

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CONTINUOUS LABORATORY REARING OF *CULISETA INORNATA* (Will.) (DIPTERA : CULICIDAE) [1]

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There is a growing need for knowledge of species of North American culicine mosquitoes that can be reared continuously in the laboratory. Potter (1951) has pointed out that there is no such thing as a standard representative test insect for studies of insecticides in the laboratory. The same applies to repellents. *Aedes aegypti* (L.), *Culex quinquefasciatus* Say, and *Anopheles quadrimaculatus* Say are the mosquitoes most commonly used in the laboratory for insecticide and repellent tests in North America. These species represent mainly the southern mosquito fauna of the continent; there has been no laboratory colony to represent the hordes of *pest* mosquitoes that infest the Canadian plains and sub-Arctic. Studies in outdoor insectaries in Western Canada have had to be made with specimens reared from larvae taken in the field or from eggs laid by captive wild females, these procedures usually being hindered by high mortalities in the resulting mosquito populations. A lack of specimens, caused by autumn frosts, has invariably interrupted studies of the biology of these important insects. Techniques have been developed for continuous rearing of the following species under laboratory conditions: *Anopheles quadrimaculatus, Anopheles punctipennis* (Say), *Aedes aegypti, Culex pipiens* L., *Culex quinquefasciatus, Aedes atropalpus* (Coq.) (Trembley, 1944, 1945); *Anopheles albimanus* Wied. (Rozeboom, 1936; Burgess, 1950); of these, only *A. punctipennis* and *C. pipiens* occur in Western Canada, and they are comparatively rare. Of some 20 species occurring in southern Manitoba, where the present work was started, only *Culiseta inornata* (Will.)—because of its stenogamous habit—showed any promise of becoming a laboratory animal.

Dupree was probably the first to observe that *C. inornata* (= *C. consobrinus* Desv.) would mate in very confined quarters (Mitchell, 1907). This fact was apparently overlooked until Owen (1937) made the same observation and suggested that this species (*Theobaldia inornata* (Will.)) would make a useful experimental animal; in a footnote he recorded his maintaining a colony of this species through five successive generations. Later (1942) he reported rearing this species continuously in the state of Wyoming, using a "semi-balanced" aquarium for the larvae. The principal ingredients of this aquarium were obtained every three months from a "vernal-autumnal" pool.

Encouraged by Owen's success, I began attempts to rear *C. inornata* in Canada in the autumn of 1942 at the Virus Laboratory of the Manitoba Department of Health and Public Welfare and the Children's Hospital of Winnipeg, Winnipeg, Manitoba, and have continued intermittently until the present. It soon became apparent that establishing a colony would be no simple problem. A balanced aquarium maintained by additions of material from a natural pool was out of the question in Manitoba. It was necessary to find an artificial medium in which the larvae would grow. During the winter of 1942–43 a small colony was maintained with difficulty for five generations, a hay infusion inoculated with paramecia being used as the larval diet. This larval rearing medium was difficult to duplicate. Numerous larval rearing media were tested during the summer of 1943, when an abundant supply of wild females was available. During the winter of 1944–45 a small colony was kept alive through

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four generations at Macdonald College, Quebec (from Manitoba stock); here the larval diet was yeast alone in water for one generation, and dried cereal grass in water until the colony died out. From August, 1946, to February, 1948, another colony was maintained at the Virus Laboratory of the Manitoba Department of Health and Public Welfare and the Children's Hospital of Winnipeg, Winnipeg, through 16 generations on a larval diet consisting of a mixture of flour and yeast in water. Here the larval mortality was variable and on more than one occasion the colony was reduced to a few individuals. During the past three years another colony was established at the Livestock Insect Laboratory, Lethbridge, Alberta, and was maintained for eight generations without signs of reduced vigor. The colony was finally allowed to die out. A routine method of rearing was developed which, I believe, will be applicable wherever the simple necessary materials and equipment are available.

C. inornata has much to recommend it as a laboratory insect. Besides its stenogamous habit, it is a large species, larger than the other North American mosquitoes that have been reared continuously in the laboratory. The male pupae are distinctly smaller than those of the females and the two sexes can readily be separated in this stage. The eggs are normally laid in the evening; but if in the evening gravid females are deprived of water they will deposit their eggs at any time during the day when placed over water, particularly against a dark background. The eggs are laid in "rafts" on the surface of the water, which facilitates harvesting. The female requires from one-half to one hour to deposit her raft. The eggs are pearly white when laid and turn dark brown in about two hours at 20° C., which simplifies the gathering of eggs of known age. The larvae can be reared in complete darkness or in light. The adults, both males and females, feed readily on almost any fluid diet presented to them; no preliminary warming of the diet is necessary to induce them to feed. The females will imbibe from a free fluid surface or through a membrane. The species is readily available in North America. It is found throughout the United States and the Mexican table land (Owen, 1942). In Canada it is found as far north as the 51st parallel in Manitoba and the 55th parallel in Alberta. Finally, *C. inornata* is a known laboratory vector of western equine encephalitis (Hammon and Reeves, 1943), St. Louis encephalitis (Hammon and Reeves, 1943a), and Japanese B encephalitis (Reeves and Hammon, 1946).

Rearing the Larvae. The greatest obstacle to the continuous rearing of *C. inornata* in the laboratory has been the lack of a suitable technique for rearing the larvae. The successful rearing of mosquito larvae in general depends on obtaining a balance between the concentration of food substances, volume and depth of medium, concentration of the larvae, and temperature. In rearing *C. inornata* it is also essential to keep the surface of the medium free of a pellicle, which if allowed to form will quickly smother the larvae.

The dietary requirements of the larvae of *Aedes aegypti* were studied by Atkin and Bacot (1917), MacGregor (1929), Trager (1935, 1935a, 1937, 1948), Trager and Subbarow (1938), Golberg and DeMeillon (1947, 1948, 1948a), and Golberg, DeMeillon, and Lavoipierre (1945) and of *Culiseta* (= *Theobaldia, incidens* (Thom.) by Frost, Herms, and Hoskins (1936). The work on these two species indicates that mosquito larvae require proteins, lipoids, vitamins of the B group, and various salts in the diet. During the present study no attempt was made to determine the specific dietary requirements of *C. inornata,* but the above information was kept in mind in seeking suitable culture media for the larvae. Nor was any attempt made to keep the cultures sterile, although all apparatus used was kept as clean as it was convenient to do.

The object of this investigation was to find a routine method for *C. inornata* that would be as simple as possible and that could be duplicated anywhere. All the

necessary dietary requirements for mosquito larvae are found in yeast, and the majority of the media recommended in the literature for mosquito larvae include yeast or yeast extracts in one form or another. Early in this investigation the methods employed by Trager (1935) (without sterilization), Woke (1937), Granett and Powers (1937), and Granett and Haynes (1944) for *Aedes aegypti,* by Phillips and Swingle (1940) for *Culex quinquefasciatus,* and by Frost, Herms, and Hoskins (1936) for *Culiseta incidens* were tried with *C. inornata.* Various modifications of their techniques were also tried, as well as media consisting of hay infusions alone or containing wheat grains or cultures of paramecia, and others containing various proprietary preparations of powdered milk and baby foods. The most helpful publications encountered were those of Trembley (1944) and Bates (1941), and the medium eventually found most satisfactory was a modification of Bates' "standard" for anopheline larvae.

C. inornata should be reared at a temperature of 20–21° C. It can be reared at 25° C. and development is more rapid at this temperature, but larval mortality is slightly higher and the adults are not as large and robust as those reared at the lower temperatures.

On being gathered, the egg-rafts were placed in enamel pudding pans eight inches in diameter and 2½ inches deep. Five to six rafts were placed in each pan, which contained a medium consisting of 100 mg. of Difco Brain Heart Infusion, 50 mg. of baker's yeast (Fleischmann's Royal), and 0.5 gm. of ground whole-wheat bread crumbs in 1250 ml. of Bates' (1941) "medium S." The bread crumbs were prepared by slicing whole-wheat bread, drying the slices in the sun or in an oven at 45° C., and then grinding them as fine as possible in a mortar. Bates' medium S consists of 0.5 gm. of calcium sulphate ($CaSO_4.2H_2O$), 0.5 gm. of sodium chloride (NaCl), and 1 gm. of magnesium sulphate ($MgSO_4.7H_2O$) in 1 litre of distilled water. A hatching pan prepared in this way accommodated about 1000 first-instar larvae. The pan was covered with a piece of window glass and a slow stream of air was bubbled continuously through the medium.

At 20–21° C. the eggs hatch in about 57 hours. The larvae were left in the hatching pans and allowed to complete their first moult there. The first moult occurs in 2–2½ days after the eggs hatch and the larvae were not transferred to the larval trays until this moult was completed and the integument had darkened. Each larval rearing tray received 150 second-instar larvae, picked up, counted, and transferred by means of a medicine dropper pipette.

The larval rearing trays were enamel instrument or photographic trays measuring 15 x 10 x 2 inches. Each tray contained 2½ litres of Bates' medium S, to which had been added 100 mg. of Difco Brain Heart Infusion, 50 mg. of baker's yeast, and 240 mg. of ground whole-wheat bread crumbs. The trays, containing these ingredients, were made up on the same day as the hatching pans, i.e., on the day the eggs were laid. These trays also were covered with window glass and air was bubbled continuously through the medium. Each second day after the trays were made up until pupation began, 50 mg. of the baker's yeast and 240 mg. of the bread crumbs were added to each tray. Since pupation began on about the tenth day after hatching, each tray received seven increments of the diet, making a total of 350 mg. of yeast and 1.7 gm. of bread crumbs.

An average of 90 per cent of the larvae pupated in five to twelve days, the majority pupating in seven days. The males began to pupate first, followed about two days later by the first female. The pupae were removed from the trays each day by means of a dipping tube and transferred to an enamel pudding pan containing distilled water through which air was bubbled continuously. The pupal pan was kept in the emergence cage.

An air stream of 1–1½ litres per hour delivered through a medicine dropper pipette to each hatching pan and larval

rearing tray was sufficient to prevent the formation of a pellicle. Too fast an air flow caused the medium to splash on the glass cover and trap some of the younger larvae. A satisfactory source of air pressure was a standard laboratory compressed-air system with a regulator to reduce the pressure to a steady five pounds per square inch or lower, depending upon the number of trays to be aerated. Also satisfactory was a laboratory blower or a Cornelius air compressor (The Cornelius Co., Minneapolis, Minn.), the compressed air being stored in a three- to five-gallon storage tank and brought up to 15–18 pounds' pressure twice a day. The outward flow of air from the storage tank was controlled by a needle valve. The beneficial effect of the air stream was probably purely mechanical, for on many occasions I have taken the larvae of *C. inornata* from pools in which there was no dissolved oxygen detectable by the Winkler method.

Bates' medium S was superior to distilled, tap, well, or pond water. In Winnipeg, tap water that had been allowed to stand exposed to the air for two days before using was satisfactory. But at Lethbridge mortality of the larvae was almost 100 per cent in media prepared with tap water. Well water and pond water gave erratic results in different localities. Difco Yeast Extract may be substituted for baker's yeast but larval mortality with the former was slightly higher. After several days the larval rearing media contained populations of bacteria and protozoa that probably originated in the bread. For some time domestic flour (Canada Approved, vitamin B) was used instead of bread, but the larval mortality varied considerably from generation to generation and this was believed due to greater variation in the numbers of microorganisms in the flour.

Feeding and Handling the Adults. Although Owen (1942) obtained a few eggs on three occasions from females fed only on sugar solutions or raisins, *C. inornata* usually requires a blood meal for the production of eggs. When mated and hungry, the females will feed readily on the human arm, either when the arm is inserted in a cage or when the females have to feed through gauze or screen as when a cage is applied to the arm. I have not been able to induce unmated females to pierce the skin for a blood meal but an occasional female will do so while in the act of mating. However, as such a method of providing a blood meal is too slow and tedious even for a colony of moderate size, a quicker and more reliable method was sought.

In studies of virus transmission by mosquitoes it is a common practice to infect the mosquitoes by allowing them to feed on a suspension of the virus soaked up in a cotton pledget. This method was adapted to feeding blood to *C. inornata*. For some unknown reason, the adults are unable to imbibe whole defibrinated, heparinized, citrated, or oxalated blood but both males and females readily take up such blood when it is diluted with a ten per cent solution of sucrose. Dilutions containing one volume of the sugar solution to six volumes or less of blood can be imbibed. The most satisfactory mixture was one volume of the sugar solution to three volumes of defibrinated blood, mortality being lowest and egg production highest on this mixture. No comparative tests were made on the blood of different species, but for practical purposes beef, pig, or sheep blood was satisfactory. A solution of the sugar in distilled water was kept on hand and added to the blood as soon as possible (one to two hours) after it was collected and defibrinated. The mixture will keep in the refrigerator at 1° C. to 5° C. for two to three weeks. For cage feeding, the blood and sugar mixture, well shaken, was soaked up by absorbent cotton held in gauze bags. Much of the blood and sugar mixture passes through the insects undigested but this also occurs when the females feed on the arm. Unmated females that have fed on the blood will produce apparently normal rafts, but the eggs in these rafts never hatch.

The emergence cage, which was also used as a feeding and egg-laying cage,

had a wooden frame 12 inches square and 22 inches high. The top and back were of three-ply wood and the sides were plates of window glass that slid in grooves in the wooden frame. The front was of unbleached cotton with a sleeve of the same material. The bottom three inches of the frame were made of 1- x 3-inch boards, which were grooved on their upper edges to receive the window glass. The bottom of the cage was an inverted galvanized iron tray with sides one inch high and perforated with nail holes one inch apart. On the inside, the bottom of the cage was covered with fine sand about one inch deep and a layer of blotting paper was placed on the sand. The cage stood in a galvanized iron pan 15 inches square and 4½ inches deep. The pan was kept filled with water to just above the level of the bottom of the cage; this served to keep the sand and blotting paper moist and maintained a satisfactory humidity in the cage. Food for the adults was provided by two gauze bags about one inch in diameter filled with absorbent cotton and soaked in the blood and sugar mixture described above. Two small gauze bags each containing four or five boiled raisins were also supplied. The four gauze bags were hung by strings about six inches long from the ceiling of the cage. The blotting paper became soiled with the faeces of the insects and had to be replaced about once a month. The growth of moulds on the blotting paper was retarded by sprinkling it with table salt. A cage of this type accommodated 1500–2000 adult mosquitoes.

At 20°–21° C. the adults emerged in about two days after pupation. The males began to emerge first, and mating took place as soon as the females emerged, sometimes before the latter were dry. Consequently it was almost certain that single females observed resting on the walls of the cage had mated.

The adults may be handled in different ways, depending on the use to be made of the colony. For maintaining the colony, the adults were left in the emergence cage and a water surface for egg-laying was provided by placing in the bottom of the cage two pans measuring 10 x 4½ x 1½ inches half-filled with water. The surface area of water provided for oviposition should be at least half that of the bottom of the cage. Under the artificial conditions in the cage the females apparently find the water surface by chance. If the free water area is too small, too many rafts are deposited on the moist blotting paper on the bottom of the cage. No air was bubbled through this water since the movement of the water surface disturbed the females resting there; instead, the water was renewed every second day. The egg-rafts were removed from the emergence cage each day. At temperatures fluctuating between 60° and 70° F., Owen (1942) obtained his first egg-rafts five to seven days after the first blood meal. At temperatures of 20°–21° C., I did not obtain the first egg-raft earlier than seven days after the first meal of the blood and sugar mixture. If a number of egg-rafts of approximately the same age were required, the females were deprived of a water surface until the tenth or twelfth day.

When confined in lantern chimney cages for experimental purposes, the adults were supplied the blood and sugar mixture soaked up in thin gauze pledgets one inch square. The pledgets were placed on top of the gauze or marquisette covering the chimney and covered with a watch glass. Each day these were renewed or alternated with boiled raisins.

The eggs of *C. inornata* can be stored for a limited time at 4°–5° C. if first allowed to darken. At these temperatures development proceeds very slowly so that when placed in the refrigerator, after aging for two to three hours at 20°–21° C., they will not hatch for about 21 days. Any time before this they can be removed from the refrigerator and will hatch normally. Temperatures of 4°–5° C. are fatal for eggs that have not yet darkened.

By this method the size of the colony that can be reared and maintained depends solely on the space and equipment available. With two constant-temperature cabinets large enough to hold three hatching pans, 14 larval rearing trays, and one

emergence cage, a colony of 1500–2000 adults can be maintained throughout the year.

Summary. A method is described for the continuous rearing of *Culiseta inornata* (Will.) in the laboratory. The larval rearing medium is a modification of one already recommended by Bates for *Anopheles* spp. Its successful application to *C. inornata* depends (1) on preventing a scum, or pellicle, from forming on the surface of the medium, (2) on rearing the first-instar larvae in a medium of high concentration, (3) on transferring the larvae only *after* the first moult is complete to a medium in which the concentration of food is gradually increased as the larvae grow, and (4) on reducing the concentration of second- and later-instar larvae to 150 in 2½ litres of the medium.

The adults are stenogamous. They feed readily and produce fertile eggs on a diet consisting of one volume of a ten per cent solution of sucrose to three volumes of defibrinated pig, beef, or sheep blood. This diet was presented to the adults soaked up in absorbent cotton or in thin gauze pledgets and required no other treatment to induce the females to feed. The blood and sugar diet was supplemented by boiled raisins.

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ABSORPTION OF RADIOACTIVE DDT BY RESISTANT AND NONRESISTANT MOSQUITOES

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The use of radioactive insecticides appears to be a promising approach to studies of absorption and distribution of the toxicant in insects. Comparing the amount of insecticide absorbed by resistant and nonresistant strains may be of value in explaining causes of resistance. Sternburg, Kearns, and Bruce (1950) and Sternburg and Kearns (1950) determined by chemical analysis the amount of DDT absorbed and some of the metabolites formed. Lindquist, Roth, Yates, Hoffman, and Butts (1951), using radioactive DDT, found that the Orlando strain of resistant house flies absorbed considerably larger amounts of DDT than did susceptible flies.

The objects of the experiments reported in this paper were to determine the amounts of DDT absorbed by resistant and nonresistant *Aedes nigromaculis* (Lud.) larvae and whether any of the DDT absorbed by the resistant larvae was degraded into other less toxic products.

TESTS WITH *Aedes vexans* AND *A. sticticus*

Tests were first made with nonresistant *Aedes vexans* (Meig.) and *A. sticticus* (Meig.) larvae and pupae to develop satisfactory technique for preparation of the material. Information on the mortality and the amounts of DDT absorbed by larvae of these species at high and low temperatures was also obtained.

Methods. Tests were made in the laboratory at 70° F. unless otherwise stated, with a mixed culture consisting of approximately 55 per cent of *vexans* and 45 per cent of *sticticus* obtained from eggs

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taken in soil near the Columbia River in Oregon. Fourth-instar larvae or pupae were used. The radioactive DDT had been prepared by random substitution of C^{14} in the benzene rings in the DDT molecule.[1]

Radioactivity was determined with a commercial type of windowless gas (pure methane) flow counter attached to a scaler.

Larvae and pupae were treated with radioactive DDT in deodorized kerosene or in acetone suspension to determine the percentage of kill and to provide specimens in which the amount of absorption could be measured with the flow counter. The kerosene solutions were applied on 700 cc. of tap water in 800-cc. beakers and the acetone suspensions on 100 cc. of tap water. From 100 to 300 pupae were used per test, and 300 larvae in all tests except in comparative series at 70° and 90° F., in which 75 larvae were used.

The kerosene preparations were applied to the surface of the water with wire loops. A small loop, delivering 0.505 cu. mm., was used in the tests with larvae and a larger one, delivering 0.742 cu. mm., in those with pupae. The loops were calibrated by comparing the radioactivity obtained from three loopfuls of the solution with that from three 1-cu. mm. drops of the material delivered by a 0.25-cc. syringe operated with a micrometer attachment. The residue clinging to the loops after application to the water surface was measured with the flow counter. There was little loss in the light applications used for larvae, but in the heavier dosages used against pupae larger amounts were withdrawn with the loop.

The mortalities and radioactivity counts from these treatments were determined after 24 hours. The larvae or pupae in these samples were either all alive or all dead. Each sample was washed for 10 to 20 seconds to remove any exterior DDT, allowed to dry, and then ground and extracted with acetone. The radioactivity was determined after the material was thoroughly dry. The results are summarized in Table 1.

Absorption by larvae. Measurable radioactivity was found in larvae treated with both the suspension and the solution of radioactive DDT after 24 hours. With both preparations the radioactivity of larvae increased as the dosage was increased.

The figures given are for the amount of radioactive DDT absorbed up to 24 hours and are not the amounts required to kill. It was found that larvae killed with chloroform gas and subjected to acetone suspensions of DDT for 24 hours absorbed approximately two-thirds as much insecticide as did living larvae. This indicates that the DDT is taken into mosquito larvae largely by absorption and that little is ingested.

Some idea of the large amount of insecticide required in an oil film as compared with the amount actually used is shown by these tests. A 54 per cent kill was obtained among 300 larvae in an 800-cc. beaker that were exposed to a DDT-oil film containing 5 μgm. of DDT on 11.78 square inches of surface. Fifty larvae, which would be a large population for such an area under normal conditions, would use only 0.16 μgm. of DDT, or about 4 per cent of the DDT present in the film.

In tests to determine the mortality of larvae and the amount of DDT absorbed at different temperatures (70° and 90° F.), it was found that in a suspension containing 0.0167 μgm the larvae held at 70° F. averaged 97 per cent mortality with an absorption of 0.0031 μgm of DDT per larva, whereas those held at 90° averaged 71 per cent mortality and the absorption was 0.0056 μgm of DDT per larva. The ability of these larvae to absorb nearly twice as much DDT at 90° as at 70° with less mortality is comparable with results obtained on house flies (unpublished data).

Absorption by Pupae. Much larger dosages of DDT were required to kill pupae than larvae. To obtain a 42 per cent kill of pupae eleven times as much

[1] Sample obtained through the courtesy of S. B. Hendricks, Bureau of Plant Industry, Soils and Agricultural Engineering.

Table 1.—Absorption of DDT by larvae and pupae of *Aedes vexans* and *sticticus*. Exposure 24 hours.

Dosage of DDT	Mortality	Living Insects: Samples tested	Living Insects: Radioactivity per sample	Living Insects: DDT absorbed per insect	Dead Insects: Samples tested	Dead Insects: Radioactivity per sample	Dead Insects: DDT absorbed per insect
	Per cent	*Number*	*C.p.m.*	*Micrograms*	*Number*	*C.p.m.*	*Micrograms*
				Tests with Larvae			
In kerosene solution (micrograms per square inch)							
0.2145	21	9	19	0.00168	4	35	0.00310
.4290	54	3	32	0.00283	9	42	0.00371
In acetone suspension (p.p.m.)							
0.0125	23	8	18	0.00194	3	16	0.00173
.0167	88	2	25	0.00270	11	20	0.00216
.025	97	–	–	–	7	30	0.00324
				Tests with Pupae			
In kerosene solution (micrograms per square inch)							
4.935	42	9	38	0.0056	4	74	0.0109
6.580	66	4	73	0.0108	8	176	0.0259
In acetone suspension (p.p.m.)							
0.2	14	3	85	0.0153	–	–	–
.5	34	2	63	0.0113	1	61	0.0110

DDT was needed as to kill 54 per-cent of the larvae. Many times as much DDT was actually absorbed by pupae as by the larvae. The ability of pupae to withstand such large dosages may be due to their power of metabolizing DDT into other products more readily than the larvae.

The dead and surviving pupae absorbed approximately equal amounts of DDT applied in acetone suspension. In the tests made with DDT in kerosene the dead pupae absorbed a little over twice as much as the living pupae. Owing to the erratic movements of the pupae that are affected by this insecticide, many parts of their bodies may touch the oil-covered surface of the water, while the pupae less affected remain in their normal position with only the breathing tubes exposed.

Aedes nigromaculis

Absorption tests. For the tests with *nigromaculis* 300 larvae were used in 200 cc. of water and all tests were made with acetone suspensions. DDT-resistant *nigromaculis* larvae from the Merced Mosquito Abatement District and nonresistant larvae from the adjoining Fresno County in California were subjected to similar treatments with radioactive DDT. The larvae were fourth instars collected from seven pastures. Gjullin and Peters (1952) reported on the studies, comparing the resistance of mosquito larvae from intensively treated areas and nontreated areas.

The data in Table 2 show that with 0.1 p.p.m. of DDT only 11 per cent of the resistant larvae were killed but that an average of 0.0325 μgm of DDT was absorbed. All the nonresistant larvae were killed, but the amount of DDT absorbed was only 0.0180 μgm per larva. The resistant larvae were therefore able to live with approximately twice as much absorbed DDT or metabolites in their bodies as were the nonresistant larvae.

The resistant larvae that died from the treatment with 0.2 p.p.m. absorbed over six times as much DDT as the nonresistant larvae treated with 0.02 p.p.m.

Bioassay of extracts of treated larvae. To determine whether the absorbed DDT

TABLE 2.—Absorption of radioactive DDT by resistant and nonresistant *Aedes nigromaculis* larvae.

DDT	Mortality	50-larva samples	Condition of larvae	Radioactivity per sample	DDT absorbed per larva
P.p.m.	*Per cent*	*Number*		*C.p.m.*	*Microgram*
		Resistant Larvae			
0.2	74	13	Dead	346	0.0345
0.1	11	20	Alive	326	0.0325
0.066	7	20	Alive	205	0.0205
		Nonresistant Larvae			
0.1	100	12	Dead	181	0.0180
0.066	100	18	Dead	123	0.01230
0.02	94	15	Dead	54	0.0054

had been degraded to a less toxic substance, extracts of *nigromaculis* larvae that had been subjected to 0.066 p.p.m. were bioassayed. Ten samples (50 larvae per sample) of resistant and nonresistant larvae on which radioactivity had been determined were prepared for bioassay using highly susceptible second-instar larvae of *vexans* and *sticticus*.

For these tests each lot was flooded with 3 cc. of acetone to dissolve the toxicant. The debris was then removed by passing the solution through a filter paper. When the filtrate had evaporated, the toxicant was re-dissolved with 0.075 cc. of acetone and 10 cc. of water was added. Twenty second-instar larvae were then added and mortality counts were made after 24 hours. Based on the radioactivity counts the resistant *nigromaculis* larvae averaged 1.14 μgm of DDT or metabolites per sample and the mortality was 7 per cent. The average for the nonresistant larvae was 0.74 μgm per sample, but the mortality was 100 per cent.

When extracts of the resistant and nonresistant *nigromaculis* larvae were tested against *vexans* and *sticticus* larvae in 10 cc. of water, they gave kills of 12 and 70 per cent, respectively. In studies of known amounts of DDT on second-instar larvae 0.005 and 0.025 μgm in 10 cc. of water gave kills of 18 and 72 per cent, respectively. In the case of the resistant larvae, therefore, the original extracts contained 1.14 μgm of radioactive materials but the bioassay showed only 0.005 μgm of DDT or other toxic substances to be in the larvae samples. This is 99.6 per cent loss of toxicity, presumably due largely to degradation of the DDT by the larvae. The nonresistant larvae showed a loss of 96.6 per cent by the bioassay method. The extracts were not bioassayed until nearly six months after preparation, and it is likely that there had also been natural deterioration of the toxic material during this time.

SUMMARY

The amounts of radioactive DDT absorbed by mosquito larvae and pupae were determined with a commercial type windowless gas flow counter attached to a scaler.

Fourth-instar larvae of *Aedes vexans* and *A. sticticus* absorbed from 0.00310 to 0.00371 μgm of DDT in 24 hours when subjected to kerosene films and from 0.00173 to 0.00324 μgm when treated with acetone suspensions of radioactive DDT. The mortalities ranged from 21 to 97 per cent. Dead larvae absorbed approximately two-thirds as much DDT as did live larvae. Larvae tested in acetone suspensions of DDT absorbed nearly twice as much DDT at 90° F. as at 70° and the mortality was lower at 90°.

Pupae of these species absorbed from 0.0109 to 0.0259 μgm in 24 hours when subjected to kerosene films and from 0.0113 to 0.0153 μgm in acetone suspensions. The mortalities ranged from 14 to 66 per cent.

Resistant *Aedes nigromaculis* larvae from pastures in Merced County in Cali-

fornia that were treated with 0.2 p.p.m. absorbed over six times as much DDT as nonresistant larvae from Fresno County that were treated with 0.02 p.p.m. Mortality of the resistant larvae was 74 per cent and of nonresistant larvae 94 per cent. At 0.1 p.p.m. resistant larvae absorbed nearly twice as much DDT as the nonresistant ones with only 11 per cent mortality as compared with 100 per cent mortality for the nonresistant group.

Bioassay of the extracts of these larvae with second-instar larvae of *vexans* and *sticticus* indicated that both resistant and nonresistant larvae had degraded a large amount of the DDT to non-toxic substances.

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OBSERVATIONS ON OVERWINTERING OF *CULEX TARSALIS* COQUILLETT (DIPTERA, CULICIDAE) IN WESTERN NEBRASKA

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The literature pertaining to the biology of the family Culicidae contain many statements to the effect that *Culex* mosquitoes overwinter as adult females in the northern latitudes of the United States. There appears to be, however, but little specific information available for individual species of this genus showing that specimens may be collected during the coldest winter months (December-March). *Culex tarsalis* Coquillett, one of the common mosquitoes in the Missouri River Basin States, is presently considered as the most important natural vector of Western equine encephalomyelitis (WEE) in the western half of the United States. As pointed out by Jenkins (1950) the question is still not answered as to whether WEE virus can survive the winter period within hibernating *C. tarsalis*. Hammon *et al.* (1945) collected mosquitoes during the winter months from the Yakima Valley, Washington, but WEE was not detected in the 18 specimens of *C. tarsalis* found and tested. In 1950–51 efforts were made to collect hibernating mosquitoes for virus examinations and to make observations on their overwintering habits in western Nebraska. Although literature references commonly state that *C. tarsalis* overwinters as the fertilized adult female and thus produces the new spring generation, no collection records for this species have been located to show that specimens may be collected regularly throughout the winter months.

Preliminary inspections to locate suitable shelters for mosquito hibernation were made during November. On November 14, 1950 several *C. tarsalis* females were

FIGURE 1—Exterior view of earthen food storage cellar 1 near Manning, Scotts Bluff County, Nebraska.
FIGURE 2—Interior view of cellar. Note maximum-minimum thermometer.

collected in an outdoor food storage cellar near Minatare, Scotts Bluff County. From two to six adult females were also collected on November 15 from each of four cellars near Whitney in Dawes County. Although many other types of structures such as barns, outbuildings of all types, and an irrigation tunnel were examined, no mosquitoes were found except in the outdoor food storage cellars. In western Nebraska these food storage cellars (figs. 1 and 2) are very common and seem to be suitable places for mosquito hibernation.

Biweekly inspections of two cellars near Minatare, Scotts Bluff County, were initiated on January 8, 1951 and were continued until the middle of May. Cellar 1 (figs. 1 and 2) was a typical outdoor cellar approximately 8 feet wide, 12 feet long, and 7 feet high with an earth cover and with the interior earth floor level approximately 5 feet below normal ground level. Cellar 2, about 8 feet wide, 9 feet long, and 6 feet high, was located beneath a farm house. This cellar had an outside entrance only. A maximum-minimum thermometer was placed in cellar 1 and temperature readings were taken on each date the cellar was inspected. These temperature readings (fig. 3), therefore, indicate the maximum and minimum air temperatures within the cellar during the period between dates of inspection. During inspections, mosquitoes were collected by means of suction tube and a flashlight. In January and February mosquitoes were stored in the cellars in quart ice cream carton cages until a number sufficient for shipment were accumulated. Only one shipment of 32 specimens was made on January 17. Eighteen of these specimens were tested for virus but the results were negative. As the winter progressed, an attempt was made to accumulate additional specimens; however, because of the high mortality of those mosquitoes kept in cages, it was not possible to make another shipment. After March 1 the use of the cages was discontinued and captured specimens were identified and again released within the cellars. There was no apparent preference for resting places inside the cellars.

Additional inspections were made during January in cellars near the towns of Whitney and Mitchell. On January 10, each of three farm cellars inspected at Whitney produced from one to three female *C. tarsalis*. During a series of inspections, four adult females were col-

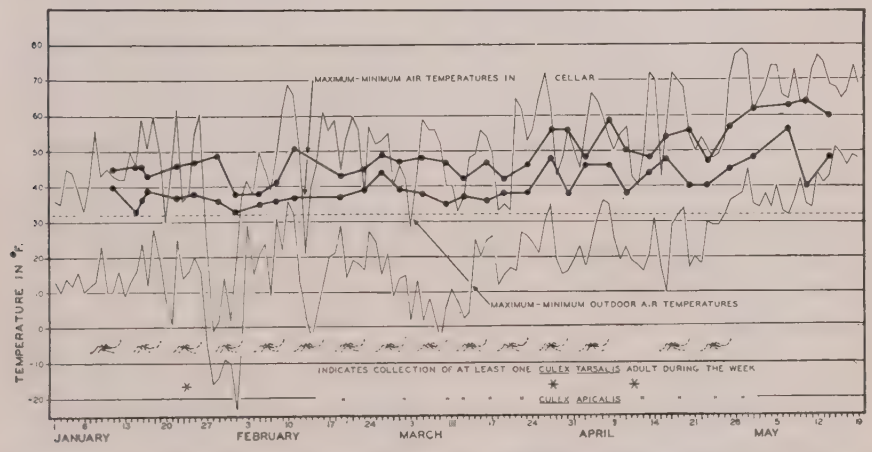

Figure 3—Maximum-minimum air temperatures in degrees Fahrenheit, January–May 1951, Nebraska. Outdoor daily temperatures by Pathfinder Irrigation District at Lake Minatare. Indoor food storage celler 1 temperatures represent periods between each inspection date at Minatare.

lected in a potato cellar near Mitchell on January 16. No male mosquitoes were found in any of the stations inspected during the winter.

Table 1 shows the number of adult fe-

TABLE 1.—Total number of adult female *Culex tarsalis* recorded weekly, January-May 1951, in two food storage cellars near Minatare, Scotts Bluff County, Nebraska.

Week Ending	Cellar 1	Cellar 2
1/13	9	3
1/20*	15	12
1/27	6	3
2/3	1	0
2/10	3	0
2/17	4	1
2/24	8	2
3/3	11	3
3/10	5	2
3/17	8	1
3/24	7	0
3/31	3	0
4/7	1	0
4/14	0	0
4/21	1	0
4/28	1	0
5/5	0	0
5/12	0	0
5/19	0	0

*Thirty-two specimens were removed on January 17 for virus examination.

male *C. tarsalis* which were found in each of the two cellars near Minatare where regular inspections were made during the winter and early spring of 1951. Because of the numerous hiding places within these cellars it was not possible at each inspection to locate all of the mosquitoes which were undoubtedly present. Until March 1 only those specimens not previously caught and placed in a cage were recorded. After March 1 some of the same mosquitoes were probably recounted at each inspection since, as already indicated, cages were no longer used after this date. Two known factors reduced the number of mosquitoes within the cellars during the observation period; these were the removal of specimens for virus studies and the mortality of those held in cages.

Maximum-minimum cellar and outdoor air temperatures are shown in figure 3. The outdoor temperatures are those recorded by the Pathfinder Irrigation District office at Lake Minatare, 8 miles north of the town of Minatare. It may be noted that the temperature within cellar 1 did not go below 33° F. at any time during the period covered by the inspections. During January and February the cellar temperatures varied from a maximum of 51° F. to a minimum of 33° F. while the outdoor air temperatures during this same period varied from a maximum of 69° F. to a minimum of —23° F. Moreover, after April 26, no outdoor air temperatures below freezing were recorded although a single record of 32° F. was made on May 7. Furthermore, after April 26, the daily maximum air temperatures were all above 60° F. and for the most part were about 70° F. or higher. It is worthy of note that after the week ending April 28, no further specimens of *C. tarsalis* adult females were found in cellar 1 which seems to indicate that in the area studied *C. tarsalis* females leave their hibernation shelters in the spring when the daily maximum-minimum temperature range fluctuates more or less continuously above the freezing point.

A light trap was operated in the vicinity of cellars 1 and 2 beginning May 15; however, no *C. tarsalis* adults were collected in this trap until June 11. Biweekly inspections for larvae were also initiated near these cellars on May 15 and the first fourth instar larvae of *C. tarsalis* were collected on May 25.

In addition to *C. tarsalis* in cellar 1, adult females of *Culex apicalis* Adams were observed on January 22, March 26 and 30, and April 9.

SUMMARY

Evidence is presented to show that hibernating *C. tarsalis* adult females may be collected in food storage cellars throughout the winter months in western Nebraska. The temperature within these cellars where specimens were regularly found did not go below 33° F. *C. tarsalis* evidently remains in hiberation until outdoor daily

minimum air temperatures are maintained above freezing.

Hibernating *C. apicalis* females were also collected on several occasions along with *C. tarsalis*.

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SOME SPECIES OF MOSQUITOES REARED FROM DRY MATERIALS [1]

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Introduction. It is well known that some mosquitoes deposit their eggs on the water surface, while others lay them in moist or dry depressions which will later be subjected to flooding. It has also been established that the eggs of those species that are not deposited directly in water are capable of withstanding considerable drying. In fact, in some cases they may remain viable after having dried for a year or more (Bates, 1949). When the depressions containing the eggs are flooded, some of the eggs present will not hatch at this time, but require subsequent drying. In most cases, the eggs which will hatch do so within a few minutes to a few hours after water is added. As a consequence of these characteristics, larvae of many species of *Aedes* and *Psorophora* can be collected in quantity only a few days after a rain. During periods of drouth they are seldom found in permanent pools.

The fact that larvae of many species of *Aedes* and *Psorophora* can be collected in large numbers only for a short time after a rain creates a problem for anyone interested in obtaining larvae during periods of drouth. Theoretically, one should be able to gather debris and soil from depressions, flood the material in the laboratory and obtain larvae of *Aedes* and *Psorophora*. So far as could be determined, however, such work has never been done on a large scale except for special situations. Dunn (1926), while investigating the breeding habits of the yellow fever mosquito in west Africa, collected dry material from tree holes and made an extensive study of the species that hatched when flooded in the laboratory. He found several species of *Aedes* common to that locality in addition to *Aedes aegypti* (L.). Wilkins and Breland (1949) recovered larvae from approximately 50% of the samples of dry material taken from tree holes in Texas. *Aedes triseriatus* (Say) and *A. zoosophus* D. & K. (= *Aedes alleni* Turner) were represented in this latter work as well as *Culiseta inornata* (Will.). Stone and Reynolds (1930) obtained specimens of *Culex, Anopheles* and *Psorophora* in Panama by flooding slightly moist material from depressions.

The Present Study. This study had

[1] Project supported by the University of Texas Research Institute.

two principal objectives. First, to discover what species of mosquitoes could be recovered from dry materials; and second, to determine whether or not this type of collecting would be a practical method of obtaining species of *Aedes* and *Psorophora* during periods of drouth.

This work was done between September, 1949, and July, 1950. Much of the material was collected within a radius of some sixty miles of Austin, Texas, but substantial numbers of samples were taken from areas considerably farther east, west and south. Since collecting mosquitoes in this way was comparatively new, the methods of collecting and treating the materials were changed from time to time as seemed necessary.

About a quart of soil and other debris per sample was collected at first, but this proved an insufficient amount and was increased to approximately a gallon, and soon to half a bushel. Small quantities were flooded in shallow procelain pans, while number three galvanized tubs were used for flooding the larger samples. The samples consisted of soil scraped from the bottom of dried depressions, tracks of animals, cracked earth and under leaves, as well as other debris in low areas subject to flooding. Leaves, grass, small twigs and partially rotted materials were also collected. All samples were dry at the time of flooding, that is, free of noticeable moisture. During the winter and early spring much of the material was damp at the time of collection. These samples were dried in the laboratory several days before flooding.

It has been established that the eggs of certain species of *Aedes* and *Psorophora* need some stimulus before hatching. This stimulus varies with the species and may take the form of certain chemicals, a change in oxygen concentration or other factors (Horsfall 1949; Bates 1949). There is still much to be learned regarding the nature and action of these stimuli, but in natural habitats they apparently occur. In the laboratory, eggs submerged in tap water often do not hatch, indicating an absence of proper stimuli or the presence of inhibiting substances. In the present work, therefore, fresh pond or river water was used. All such water was strained through cloth to remove possible eggs or larvae that might be present. Pans and tubs were kept covered to avoid contamination by female mosquitoes in the laboratory.

As has been previously stated, all eggs in a single batch of material often do not hatch at the same time; therefore, some samples were flooded several times with a long period of drying between each flooding. This practice was usually not carried out with the large samples because the bulk would have required more space for drying than was available. However, several small samples of soil taken from edges of ponds and other places subject to flooding, such as tree holes and rock holes, were treated in this way.

DISCUSSION. For this study 195 samples of dry materials have been collected and flooded. A total of 5 genera of mosquitoes, including approximately 22 species, were recovered. Some species could not be determined with certainty, because only one or a few specimens were obtained, and these either died before attaining the fourth larval instar, or were in poor condition for determination. The results of this work are summarized below. A few additional observations relative to specific species may be of interest.

It has been noted previously that some samples were flooded several times with periods of drying between floodings. Samples taken from tree holes usually yielded more larvae when reflooded than other samples. From one sample of tree hole debris to which water was added thirteen times, *Aedes triseriatus* (Say) was recovered twelve times and *Aedes zoosophus* Dyar and Knab five times.

In an effort to obtain egg deposition, fifty adult *Aedes sollicitans* (Walker), reared from dry material were placed in a cage measuring approximately 18 x 18 x 18 inches. The cage was covered with wet burlap bags to increase the humidity, and dishes of grass and mud dampened with sea water were supplied. Pieces of

oranges and apples were placed in the cage for the males, and through sock openings females fed freely on one of the writer's arms. Mortality was much higher among males than females, and all of the males were dead within 15 days. Several females survived for more than 40 days, but all except one were dead on the forty-fifth day. This lone female attained an age of fifty-six days before she died. Egg deposition apparently did not occur, since no larvae were recovered, although the debris in the dishes were flooded and dried several times.

Perhaps the most significant fact connected with this work was the recovery of several larvae of *Culex, Anopheles* and *Culiseta,* as described below. During this time egg rafts of *Culiseta inornata* (Williston) were easily collected in quantity in the field and experiments were designed to test the reaction of the eggs to drouth and freezing temperatures.

SYNOPSIS OF DATA ON MOSQUITOES RECOVERED FROM 195 SAMPLES OF DRY MATERIALS (September, 1949 to July, 1950). 1. *Aedes aegypti* (Linn.): Austin, Travis County; from soil in an old bucket, 1 sample yielded larvae, 5 recovered. 2. *A. sollicitans* (Walker): Corpus Christi, Nueces County, Copano Bay, Aransas County; from salt marsh grass, 5 samples yielded larvae, 550 recovered. 3. *A. atropalpus* Coq.: Junction, Kimble County, Del Rio, Val Verde County; from soil and sand in rock holes, 2 samples yielded larvae, 150 recovered. 4. *A. vexans* Meig.: Palmetto State Park, Gonzales County; from leaves, soil and rotted debris, 6 samples yielded larvae, 200 recovered. 5. *A. infirmatus* D. & K.: Palmetto State Park, Gonzales County; from leaves and soil, 1 sample yielded larvae, 4 recovered. 6. *A. triseriatus* (Say): Junction, Kimble County, Huntsville, Walker County, Livingston, Polk County, Woodville, Tyler County; from rotted wood from tree holes, 8 samples yielded larvae, 100 recovered. 7. *A. zoosophus* D. & K.: Junction, Kimble County; from rotted wood from tree holes, 1 sample yielded larvae, 40 recovered. 8. *A. trivittatus* (Coq.): Junction, Kimble County; from drift material, 1 sample yielded larvae, 13 recovered. 9. *A. thelcter* Dyar: Palmetto State Park, Gonzales County; from leaves and soil, 2 samples yielded larvae, 8 recovered.

10. *Psorophora ferox* (Humboldt): Austin, Travis County, Palmetto State Park, Gonzales County; from leaves, drift and soil, 6 samples yielded larvae, 25 recovered. 11. *P. longipalpus* Roth: Palmetto State Park, Gonzales County; from leaves, drift and soil, 3 samples yielded larvae, 25 recovered. 12. *P. horrida* (D. & K.):* Palmetto State Park, Gonzales County; from leaves and surface soil, 1 sample yielded larvae, 6 recovered. 13. *P. cyanescens* (Coq.): Copano Bay, Aransas County; from *Andropogon* and marsh grass, 1 sample yielded larvae, 10 recovered. 14. *P. signipennis* (Coq.): Junction, Kimble County, Palmetto State Park, Gonzales County; from leaves, grass and trash, 2 samples yielded larvae, 55 recovered. 15. *P. confinnis* (Lynch Arribalzaga): Raymondville, Willacy County, Palmetto State Park, Gonzales County; from grass, leaves and soil, 3 samples yielded larvae, 12 recovered. 16. *P. ciliata* (Fab.): Williams Creek, Travis County, Palmetto State Park, Gonzales County; from leaves and surface soil, 6 samples yielded larvae, 50 recovered. 17. *P. howardii* Coq.: Williams Creek, Travis County, Palmetto State Park, Gonzales County; from leaves and surface soil, 6 samples yielded larvae, 56 recovered. 18. *P. varipes* (Coq).:* Palmetto State Park, Gonzales County; from leaves and surface soil, 1 sample yielded larvae, 2 recovered.

19. *Culex restuans* Theob.: Williams Creek, Travis County; from leaves and surface soil, 1 sample yielded larvae, 3 recovered. 20. *C. salinarius* Coq.: Palmetto State Park, Gonzales County; from leaves and surface soil, 1 sample yielded larvae, 2 recovered.

21. *Culiseta inornata* (Williston): Williams Creek, Travis County, Palmetto State Park, Gonzales County; 6 samples yielded larvae, 14 recovered.

* Of doubtful identity.

22. *Anopheles pseudopunctipennis* Theob.:* Gaines Ranch, Travis County, Williams Creek, Travis County; from leaves and surface soil, 2 samples yielded larvae, 4 recovered.

Culiseta inornata normally lays its eggs in the form of rafts on the water surface. In the southern part of the United States, *C. inornata* tends to be a winter mosquito, and in this area is usually quite common from October or November until March or April. In the north, the species breeds in the spring and summer. It is probable that this mosquito passes the winter by hibernating in the adult state in the North (Matheson 1946), but it is not known how the species survives the summers in the South (King, Bradley and McNeel 1944).

In the vicinity of Austin, Texas, Wilkins and Breland (1949) reported having collected material from a dry tree hole, which was known to have been free of water for at least two months. From this sample they obtained more than fifty larvae of *C. inornata.* They suggested that at least part of the population in this area passes the summer in the egg stage.

The writer performed two experiments with egg rafts of *Culiseta inornata* in an effort to elucidate this problem further. Twenty rafts were used in the first and 200 rafts in the second experiment. The 20 rafts were divided into 4 groups of 5 rafts each, and the 200 rafts were divided into 10 groups of 20 rafts each. Moist, well rotted leaves were placed in each of a number of small dishes. Seven of these egg groups were placed beneath the surface of the leaves, and the remainder, except for one group of 20 rafts flooded at once as a control, were placed on the surface of the leaves in dishes.

The control group of 20 rafts hatched within a short time after being placed in water. The percentage of hatch, insofar as could be determined, was close to 100 per cent. The experimental rafts, both those exposed to the air and those covered by leaves, were subjected to the same types of treatment. These included exposure to laboratory temperatures, freezing temperatures and temperatures from five to fifteen degrees centigrade. None of the rafts hatched that were on the surface of the leaves. However, many of those that were covered by leaves hatched, in some cases yielding a high percentage of larvae. The specific treatment given each group of eggs, and the approximate percentage of eggs yielding larvae, are noted below.

The approximate percentage hatch of *Culiseta inornata* eggs under moist leaves exposed to different conditions was as follows: (1) 20 rafts exposed to outside temperature for 36 hours, lowest temperature 5° C.: 75 per cent hatch. (2) 5 rafts exposed to room temperature for 72 hours, average temperature about 27° C.: 50 per cent hatch; 20 rafts under same conditions, 75 per cent hatch. (3) 20 rafts exposed to room temperature (average 27° C.) for 72 hours; then 5° C. for 24 hours, 98 per cent hatch. (4) 5 rafts exposed to room temperature (average 27° C.) for 72 hours, then —8° C. for 24 hours, 90 per cent hatch; 20 rafts under same conditions, 98 per cent hatch. (5) 20 rafts exposed to room temperature (average 27° C.) for 72 hours; then —8° C. for 48 hours, 98 per cent hatch.

These results indicate that the eggs of *C. inornata* are capable of surviving for a time in the absence of free water if the rafts are covered by leaves. It is also obvious that under the same conditions the rafts will withstand freezing temperatures for a time. The percentage of eggs that hatch, however, is actually no accurate criterion of the number of larvae that are likely to complete their development. Larvae that hatched from all groups of eggs that were exposed to freezing, began to die shortly after emerging. Approximately 50 per cent of the larvae from eggs exposed to minus 8 degrees centigrade for 24 hours survived. The larvae from eggs kept at this temperature for 48 hours all died within a few hours, an indication that the ability of the eggs to withstand freezing is a limited one.

One of the principal questions that

* Of doubtful identity.

arises is whether the *Culex, Culiseta* and *Anopheles* survived in the egg or larval stage. The writers are unable to answer this question at the present time. No larvae of these genera were observed in the first instar but all were discovered in the tubs in second, third and fourth stadia. This fact is not especially significant, however. When dry material is flooded, so much debris often floats on the water surface that first instar larvae are easily overlooked if only a few specimens are present.

Stone and Reynolds (1939) recovered first instar larvae of *Culex* and *Anopheles* by flooding material that was only slightly moist. They suggested that the *Anopheles* at least had survived in the egg stage. This same suggestion has also been made in the case of *Culiseta* by Wilkins and Breland (1949). Survival in the egg stage is supported by the fact that in the present work, eggs remained viable for a time in the absence of free water.

Under the conditions outlined, the writers do not feel that routine collecting of dry material is a practical method of obtaining mosquito larvae. In many cases, few or no specimens were recovered, even though half a bushel or more material was collected. Percentage of recoveries was much higher, however, from debris collected from tree holes. This fact has been previously confirmed (Wilkins and Breland 1949). Also, a relatively small amount of tree hole debris may yield a large number of larvae. These facts indicate the practicability of obtaining species of *Aedes* from tree holes by collecting dry samples.

It appears probable that other species with a limited breeding habitat, such as salt marsh and rock hole breeders, could be collected profitably by this method. It is also suggested that a higher percentage of recoveries could be obtained by flooding damp material immediately rather than by allowing it to dry several days before flooding.

Other Invertebrates. In addition to the species of mosquito larvae obtained in this work, a number of other invertebrates were also recovered. Inasmuch as it was not the objective of this study to investigate invertebrates other than mosquitoes, only the most prominent forms were noted. No attempt was made to identify the species, but an effort was made to place the most numerous ones in the proper order, family or genus.

Numerous Protozoa were observed in nearly every flooding of material, *Paramecium* being the most common. Many Oligochaetes were obtained from about seventy-five per cent of the samples. Those noted particularly were *Lumbricus* and *Aulophorus*. Several Crustaceans were recovered including *Daphnia* (Cladocera), *Cyclops* (Copepoda), fairy-shrimp, probably *Streptocephalus texanus* (Anostraca), *Apus* (Notostraca), and one or more species of Conchostraca.

Members of six families of insects were noted including Chironomidae; Heleidae, probably *Culicoides;* Syrphidae, possibly of the genus *Eristalis;* Psychodidae, possibly of the genus *Psychoda* (Diptera); Belostomatidae (Hemiptera), and Dytiscidae, possibly *Dytiscus* (Coleoptera). The Belostomatidae and Dytiscidae were a half centimeter or more in length when first observed, therefore, it is suspected that these insects survived drouth in the debris as nymphs and larvae instead of hatching from eggs.

Summary. 1. A study has been made of the species of mosquitoes reared from 195 samples of dry material flooded with water in the laboratory. Larvae were obtained from 58 samples.

2. Some observations were also made upon the laboratory reactions of *A. sollicitans* reared from dry material, and upon the ability of egg rafts of *Culiseta inornata* to withstand drying and freezing.

3. Five genera of mosquitoes were recovered, including nineteen species positively identified and three tentatively identified. These included species of *Aedes, Psorophora, Culex, Culiseta,* and *Anopheles*. Many other invertebrates were also obtained.

4. It is not known in what stage the few specimens recovered of *Culex, Culi-*

seta, and *Anopheles* survived. The fact that egg rafts of *Culiseta inornata,* if covered by leaves, will withstand a certain amount of drying suggests that it was the egg stage.

5. Less than one-third of the samples collected yielded larvae, and in many cases only a few specimens were obtained. Under the conditions outlined, it is thus not believed that routine collecting of dry material is a practical method of obtaining mosquito larvae.

6. A relatively high percentage of dry debris obtained from tree holes yielded larvae when flooded in the laboratory. These results have also been confirmed by the writer's associates. It is thus believed that collecting dry debris is a practical method of obtaining certain tree hole breeding species. It is also suggested that other mosquitoes with a limited breeding habitat might be recovered in quantity by the collection and flooding of dry material.

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A SMALL-ANIMAL RESTRAINER FOR FEEDING MOSQUITOES IN SMALL CAGES

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A cheap, easily constructed, small-animal (guinea pig) restrainer for feeding mosquitoes in small experimental cages (10 x 10 x 10″) has proved so successful and so easily handled in this laboratory that it might also be profitably employed by other workers. Essentially this restrainer is a modification and refinement of one described by Heal and Pergrin (1945). different size. Four thick wire bows (L) attached to the sides of the wooden base arch over the space between the housings, leaving ample feeding space for mosquitoes. In addition, the restrainer contains at one end a metal head-restraining bow to prevent the animal's head from protruding. The new features of this restrainer are the rack and door housings,

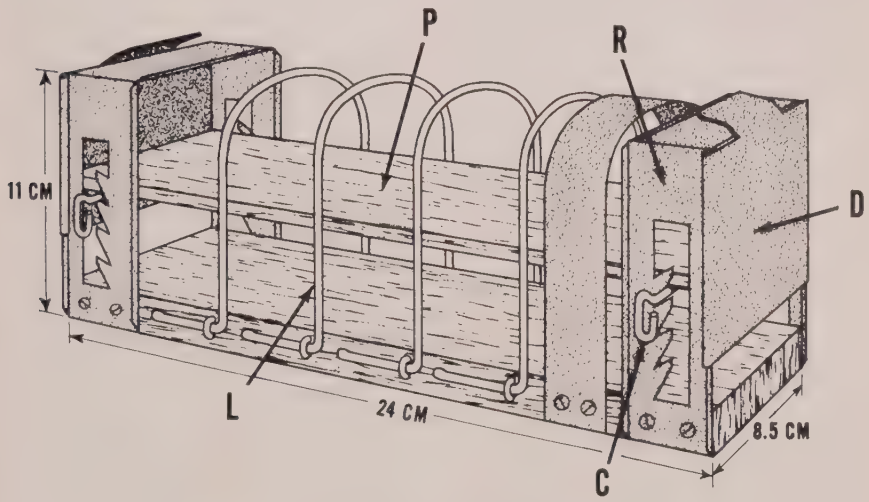

FIGURE 1.—Restrainer for feeding mosquitoes. C, rack bar; D, sliding door; L = wire bow; P, platform; R, rack and door housing.

The restrainer (figure 1) consists of a wooden base, 24.0 x 11.0 x 2.0 cm., and two metal rack and door housings (R) attached by screws to the ends of the wooden base. These housings support sliding metal doors (D). The racks, cut into each of the two sides of the housings, have movable rack bars (C) which support an elevating platform (P) that can be easily adjusted to confine animals of movable platform, and head-restraining bow.

This entire restrainer can be made from tin cans, coat hanger wire, and portions of orange crates. Such a restrainer might be modified for holding rabbits or other experimental animals.

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MICROINJECTION TECHNIQUE IN MOSQUITOES [1]

GEORGE F. SHAMBAUGH [2]

Microinjection as a technique is widely used in toxicology and physiology. It has been used in the study of insect hormones by Day and Powning (1949) and Fisk and Shambaugh (1952). The technique would lend itself to studies of insect-borne diseases, such as malaria.

For this technique two pieces of apparatus were constructed: a mouth syringe and a mosquito holder. The mouth syringe consisted of a small rubber tube 15 cm. long with a plastic mouth piece from a haemocytometer unit. The rubber tube was attached to a 6.5 cm. piece of 3 mm. inside diameter glass tubing. The tubing was drawn to an abrupt capillary point, 0.2 mm. in diameter. The tube was coated inside and out with paraffin as described by Wigglesworth (1937).

The holder (Fig. 1) is constructed of a piece of glass tubing 9 cm. long and 3 mm. inside diameter. A wood block 1 cm. long was placed into one end of the tube. An insect pin, #0, was forced between the wood and the side of the tube. The head of the pin protrudes 3 mm. A small piece of nichrome wire was forced between the wood and glass on the opposite side. The wire extends beyond the end of the tube approximately 6 mm. The two wires extend beyond the wood inside the tube. These are held securely by paraffin.

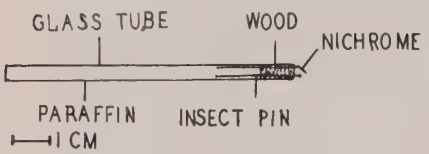

FIGURE 1. A diagram of an apparatus to hold adult *Aedes aegypti* mosquitoes during injection.

The nichrome wire was bent at an obtuse angle, so that the distance between the head of the insect pin and the wire is slightly less than the width of the thorax of the mosquito (0.7 mm.). The two wires were slid over the thorax of an anesthetized mosquito. The mosquito could then be manipulated without danger of crushing it. Although the holder was designed by the author for use on *Aedes aegypti* females, it undoubtedly could be modified for use on other species.

Mosquitoes to be injected were anesthetized with CO_2 and picked up in the holder. Under the field of a dissecting microscope the tip of the syringe containing a given solution was inserted into the thorax between the metapleuron and mesopleuron. The solution was forced posteriorly so that the abdomen becomes noticeably distended.

The mosquitoes seemed to be in a state comparable to shock after injection. Each mosquito was placed in a shell vial until later dissection. With a little practice no mortality was experienced due to the injection.

Literature Cited

DAY, M. F. and R. F. POWNING. 1949. A study of the processes of digestion in certain insects. *Austral. Jour. Sci. Res.* (ser. B) 2: 175–215.

FISK, F. W. and G. F. SHAMBAUGH. 1952. Protease activity in adult *Aedes aegypti* mosquitoes as related to feeding. *O. Jour. Sci.* 52(2):80–88.

WIGGLESWORTH, V. B. 1937. A simple method of volumetric analysis for small quantities of fluid: estimation of chloride in 0.3 microliters of tissue fluid. *Biochem. Jour.* 31:1719–22.

[1] This study was supported in part from funds granted by The Ohio State University Research Foundation to the University for aid in fundamental research.

[2] Research fellow in the Department of Zoology and Entomology at The Ohio State University.

ASSOCIATION NEWS

An informal "summer field trip meeting" of as many AMCA members as could assemble from the northeastern area was held at Ottawa, Canada, on July 30 and 31. President C. R. Twinn and his associates in the Science Service, Canada Department of Agriculture, and the Ottawa Federal District Commission acted as hosts of the Association.

The meeting included a trip to the Dominion Experimental Farm. Here, opportunity was afforded to inspect the new building which is designed especially to house biological and insecticide studies on insects affecting man and animals, and to visit Dr. Twinn's headquarters in the Science Service Building.

In the Science Service Building projects of special interest in Dr. Twinn's department were the fly resistance work and repellency studies. Mr. Lloyd Roadhouse demonstrated the technique used in testing chemicals for possible repellency to mosquitoes, with guinea pigs as the test animals. These are used particularly for screening compounds that may possibly be toxic.

Another interesting set of experiments was described by Dr. F. J. O'Rourke, medical entomologist, formerly of the Liverpool School of Tropical Medicine, England. He and Dr. A. S. West, of Queen's University, are doing serological studies which, through tests analogous to complement fixation tests, can reveal what animal a mosquito has been feeding upon, or as Dr. West mentioned later in connection with the motion picture, the tests are designed to reveal "what feeds on what."

Demonstrations of fogging and spraying equipment were held near Hull, in Quebec, and a visit was made to a floodwater mosquito-producing area along the Ottawa river. In the spring the river at this point rises and sometimes overflows the surrounding land which includes a considerable residential area. Several islands in the river are also partially under water at this time. When the water recedes, large areas of potential mosquito-breeding water are left. These are larvicided by airplane, and also sprayed or fogged for the elimination of adults, if necessary.

The AMCA members had luncheon at the Chez Henri in Hull at noon, and were guests of the Federal District Commission at dinner at the Chateau Laurier in the evening on July 30.

After the dinner an excellent motion picture of biting-fly studies in northern Canada was shown. This is the same film, with recent additions, that was shown at Salt Lake City. It includes views of northern *Aedes spp.* swarming around the investigators, and of the planes used to eliminate these mosquitoes; blackfly streams with blackfly larvae in their normal attachment sites; closeup views of *Tabanus spp.* biting; and some of the protective clothing worn by persons who have to frequent the northern tundra and forests. Some of the apparatus used in the studies was also shown. Of especial interest was a net which could be slid along a wire, turned around, and pulled back and forth, all at a sufficient distance from the operator so that the flies caught were captured on the wing in normal flight, uninfluenced by the potential host.

On July 31 a business meeting was held during a picnic trip at Philips's Lake in the Laurentian hills. On this trip the AMCA members were again the guests of the Federal District Commission.

Although the "business meeting" was officially unofficial, several important matters were discussed, among them the problem of a successor to Bob Vannote whose many services to the Association have multiplied to the extent that he feels compelled to ask to be relieved of the Advertising Managership of *Mosquito News*.

The publication of Helen Louise Trembley's paper on mosquito rearing techniques as AMCA Bulletin No. 3 was discussed, and Mr. Vannote, as chairman of the Committee on Miscellaneous Publications, was asked to secure information on costs.

The "business meeting" actually consti-

tuted only a discussion which, in the absence of a majority of directors, could only take the form of suggestions and advice to the President, to the Editor of *Mosquito News,* and to the Chairmen of Committees. For this purpose such informal regional meetings of as many members as can assemble in an area are invaluable aids to the officers and directors in promoting the activities of the Association as a whole, and those responsible for conducting the business of the Association can take official action later on the suggestions emanating from such meetings whenever and wherever held.

The photograph below shows most of those who attended the Ottawa meeting. Others who attended but who do not appear in the photograph were F. P. Creadon of Riverside, Ill., Walter Blank of Poughkeepsie, N. Y., and Messrs. R. L. Twinn, D. G. Peterson and C. Beddoe of Ottawa and S. Holme of Minehead, England. Also present were Mrs. Twinn, Mrs. Blank, Mrs. Austin, Mrs. Williamson, Mrs. Roadhouse, Mrs. O'Rourke, Mrs. Creadon and Mrs. Holme.

In conclusion, the AMCA, through *Mosquito News,* conveys its warmest appreciation to the Federal District Commission for its hospitality, and also to the Ottawa newspapers, the *Evening Citizen* and the *Journal* which, by extensive, accurate (and at times facetious) reporting and editorial comment gave fine and favorable publicity to the AMCA and its work.

Some of those present at the Ottawa summer field trip of the A.M.C.A., July 30–31, 1952. (Left to right) *Front Row:* H. Jamnback, W. V. Bartosewitz, R. Glen, H. H. Stage, C. R. Twinn, F. C. Bishopp, R. L. Vannote, L. W. Smith, C. T. Williamson, P. B. Brockway Jr., A. C. Jones. *Back Row:* H. F. Austin, Don L. Collins, Otto McFeely, H. G. James, R. R. Hall, L. E. Hagmann, J. F. Sharp, A. W. A. Brown. L. A. Roadhouse, A. S. West, V. Conant, F. J. O'Rourke, J. W. Scott, W. M. Askwith.

AMCA DAYTONA-WAY

The next annual meeting of the American Mosquito Control Association will be held jointly with that of the Florida Association at Daytona Beach, Florida, in April of 1953. The dates: April 12 (Sunday) through April 16 (Thursday). The place: the Sheraton Plaza Hotel, on the ocean at Daytona Beach. Easter Sunday comes on April 5, which might be a good starting date for those making the trip from a distance.

Sunday, the 12th, will be taken up by registration, a social hour and any special Directors' meetings that may be called. Monday and at least half of Tuesday will be devoted largely to the contributions of the Florida Association. The rest of the time will be occupied by the general sessions and papers, and such field trips and special events as may be planned.

Dr. Fred C. Bishopp is chairman of the Program Committee, and an outstanding list of speakers and papers is thus assured.

Among the events planned are a visit to the Bok "Singing Tower," the Cypress Gardens and Silver Springs on Friday and Saturday, ending with a trip to Miami on Saturday evening. Trips to the Orlando Laboratory of the U. S. Department of Agriculture and to other research centers will also be scheduled, as well as visits to local mosquito control projects.

Sam Minnich, chairman of the local committee who has given out the above information, adds, "We are really getting up this joint meeting for a bang-up session with plenty of entertainment in between the working sessions, with a dance banquet, top speakers, and an M.C."

More details of the program will be circulated when they become available. In the meantime, brief your organization on the importance of these meetings and the necessity of attending.

NOTICE TO PARTICIPANTS IN ANNUAL MEETING

If you wish to present a paper at the annual meeting at Daytona Beach (April 12–16, 1953), please complete the form on the other side of this notice, and send it to Dr. F. C. Bishopp, Chairman of the Program Committee, AMCA, U. S. Bureau of Entomology and Plant Quarantine, Department of Agriculture, Washington 25, D. C., not later than February 1.

The reading of most papers should require not more than 10 minutes. Before preparing lantern slides for use in connection with the reading of a paper, it is strongly urged that the contributor read "Suggestions . . . with Regard to Projection Equipment and Its Use," by a special committee of the American Association of Economic Entomologists, in the *Journal of Economic Entomology,* Vol. 44, No. 1 (Feb. 1951), pp. 139–140.

The suggestions in the report cited can be condensed to two principal rules:

1. Do not crowd too much on one slide.
2. Make original drawings with bold lines and large letters and figures.

Except on "radio mats" or their equivalent where direct projection is possible, typing is not recommended for slides; it will not stand reduction and the necessary enlargement. Use not more than 30 letters and spaces horizontally, not more than 10 lines vertically. Leave ample space around words, figures and characters. Many tables which can be followed easily on a printed page cannot be followed on a slide and must be condensed, or have portions reproduced on separate slides.

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(To be filled out, detached, and returned as directed on the other side of this form)

Title of paper to be presented at the annual meeting of the AMCA in Daytona Beach on April 12–16, 1953.

Title .....

.....

Author(s) .....

Institution .....

Address .....

Facilities needed (underscore those needed): Standard lantern (3¼ x 4), Opaque projector, Motion picture projector (16 mm), 2 x 2 slide projector.

It is requested that you submit an abstract of your paper to the chairman of the program committee not later than March 1.

NEWS AND NOTES

The First Inter-American Congress of Public Health will be held at Havana, Cuba, from Sept. 26 to Oct. 1, 1952, pursuant to a resolution of the XIII Pan American Sanitary Conference. This official meeting will serve to commemorate the fiftieth anniversary of the Pan American Sanitary Bureau and to pay homage to Carlos J. Finlay.

The Congress is being organized jointly by the Government of the Republic of Cuba and the Pan American Sanitary Bureau. The importance of the topics to be considered at the plenary sessions, symposia and round tables, as well as the scientific prestige of the experts who will participate in the discussions, are indicative of the significance of this congress in the development of public health in the Americas.

The American Mosquito Control Association has been invited to send delegates to this congress. President C. R. Twinn has named F. C. Bishopp and H. H. Stage to represent the Association.

The Fifth International Congresses of Tropical Medicine and Malaria will be held in Istanbul, Turkey, from Aug. 28 to Sept. 4, 1953. Reports and papers must be sent to the Secretary-General, Prof. Dr. Ihsan Sukru Akael, Tunel Meydani, Beyoglu, Istanbul, Turkey, before Feb. 1, 1953.

Marston Bates has joined the faculty of the University of Michigan as Professor of Zoology. Dr. Bates, who has been with the Rockefeller Foundation since 1937, is well known for his research on mosquito biology and for his book, "The Natural History of Mosquitoes."

The East Side Mosquito Abatement District has built two foggers mounted on trailers. The fog unit is an "Insect-O-Fog" using butane fuel. The normal operating temperature at the fog head is about 1200 degrees. The maximum discharge is about 100 gallons per hour, but settings producing 30 to 60 gallons per hour are generally used. Using one gallon of 20% lindane to 100 gallons of no. 2 diesel oil, the control achieved with this machine has been very good.

In the July number of "Mosquito Buzz" there is a description of alterations that can be made on the brake system of the Willys Jeep that give more braking power, reduce pedal pressure, and result in longer life to drums and shoes.

C. M. Gjullin and A. W. Lindquist are continuing their studies on radioactive DDT at the Bureau of Entomology and Plant Quarantine laboratory in Corvallis, Oregon. They have recently reported that larvae of resistant mosquitoes absorbed more than six times as much radioactive DDT as nonresistant larvae of the same species, yet 20% fewer resistant larvae died. (See paper in this issue of the *News*.)

The First Annual Meeting of the American Society of Tropical Medicine and Hygiene will be held jointly with the American Academy of Tropical Medicine at the Galvez Hotel, Galveston, Texas, Nov. 13 to 15, 1952. A feature of the meeting will be a symposium on "Sanitary Engineering in the Tropics." Included in the program will be clinical, field, and research papers representing the broad field of tropical medicine and hygiene—medicine, nursing, engineering, malariology, entomology, parasitology, etc. Further information may be obtained from Quentin M. Geiman, Secretary-Treasurer, The American Society of Tropical Medicine and Hygiene, Harvard School of Public Health, 25 Shattuck St., Boston 15, Mass.

Dr. John A. Rowe has been named Assistant Chief of the Water Projects Section of the Communicable Disease Center. The new section has headquarters in Salt Lake City. It will cooperate with Federal water development agencies and State Health Departments in the control of insects of public health significance associated with the development of the nation's water resources. Mosquito reduction, in conjunction with conservation irrigation, forms one of the principal goals of the CDC program which will extend into 17 western states.

Dr. Fred C. Bishopp was honored by his alma mater, Colorado A. and M. College, at the June commencement exercises. He received a plaque in recognition of his outstanding scholastic and professional achievements. A graduate of the class of 1902, Dr. Bishopp joined the Bureau of Entomology of the U. S. Department of Agriculture in 1904. He was made Chief of the Division of Insects Affecting Man and Animals when it was created in 1926 and in 1941 was appointed to his present post of Assistant Chief of the Bureau, in charge of research. He received his Ph.D. degree from Ohio State University in 1932.

The Entomological Society of America, as a result of a second vote, will consolidate with the American Association of Economic Entomologists, effective Jan. 1, 1953. The new organization which will be known as the Entomological Society of America has as its purpose the promotion of the science of entomology in all its branches. The membership will be grouped in six sections as follows: General Entomology; Insect Physiology and Toxicology; Insect Biology; Medical and Veterinary Entomology; Insect Control, Survey, and Regulatory Entomology; and Chemical Control Investigations.

Publication of the Annals of the Entomological Society of America and the Journal of Economic Entomology will continue. It is proposed to publish a monthly Bulletin to include items of current and timely interest.

EDITORIAL SECTION

MOSQUITO CONTROL

It is unnecessary in the pages of this journal to emphasize the importance of mosquitoes and other blood-sucking flies to the health and welfare of man and to the well-being and productive capacity of his livestock. The necessity or desirability of controlling them is fully recognized by members of the A.M.C.A. The effective control of these insects, however, is not simple, easy, nor as inexpensive as many people apparently believe. On the contrary, mosquito control in many areas is, in fact, a complex, difficult problem the satisfactory solution of which requires the application of knowledge in biology, chemistry, and associated sciences and of engineering and other skills, supported by adequate funds and organization.

The main objective of the American Mosquito Control Association, as set forth in the Bylaws of the Association (MOSQUITO NEWS, 10:220–227, 1950) is to disseminate information about mosquitoes and their control and to work for the highest standards of efficiency in such work, and to encourage further research toward this end. The Association's journal, MOSQUITO NEWS, has contributed very materially to the realization of these objectives. Another activity of the Association that is making important contributions is the publication of a series of bulletins designed to provide complete, up-to-date information on the methods, materials, and equipment used in mosquito control. Two of these bulletins have been published and widely disseminated. No. 1, issued in March, 1948, dealt with "The Use of Aircraft in the Control of Mosquitoes"; No. 2, published in March, 1952, has to do with "Ground Equipment and Insecticides for Mosquito Control." No. 3, which deals with "Mosquito Rearing Techniques," is under preparation.

The sale of Bulletin No. 2 appears to be initially rather slow, probably because it has not been sufficiently publicized. However, it is anticipated that there will be an increasing demand for it as it becomes more widely known. This bulletin is well written, usefully illustrated, up-to-date and authoritative, and reflects great credit on Mr. Robert L. Vannote and the members of his Miscellaneous Publications Committee who prepared it, and on the editor, Dr. E. F. Knipling. It is a mine of information essential to all concerned with the control of mosquitoes and other biting flies, and should also prove valuable in the general field of insecticide application. In the words of Dr. Knipling: "unless the bulletin has been studied . . . I am sure those who are actively engaged in control work will not realize the amount of information it does contain which may be of help to them. Therefore, I feel . . . that members of organized control districts should have copies. . . . The publications committee were hopeful that many of the control organizations would purchase copies for their employees. Local health departments in cities and towns should be advised of the availability of this publication, for it should prove a very useful reference and guide for emergency insect problems which constantly arise. The publication was edited with the thought in mind that it would be useful not only to workers in our countries, but to mosquito control workers in any part of the world. I believe that it would be worthwhile to make the publication known to control agencies outside of the United States and Canada."

Members of the Association are urged to make a special effort to bring this fine bulletin to the attention of interested organizations and individuals with a view to increasing and speeding up its sale and distribution. This would be both a public service and a help to the Association.—C. R. TWINN.

REVIEWS AND ABSTRACTS

Yellow Fever. George K. Strode, Editor, and John C. Bugher, J. Austin Kerr, Hugh H. Smith, Kenneth C. Smithburn, Richard M. Taylor, Max Theiler, Andrew J. Warren, and Loring Whitman. Published by McGraw-Hill 1951, 710 pages, over 600 ref. This book has presented a fascinating story of one of the undertakings of The Rockefeller Foundation in the world-wide campaign aganst yellow fever from 1916 through 1949. During this period the Foundation contributed over 13 million dollars, and 76 scientists served a total of 465 man-years. The largest expenditure, over 5 million dollars, was made in Brazil.

There are 10 chapters, each prepared by one of the authors (Bugher 2), and editor; 1, Landmarks in the Conquest of Yellow Fever; 2, The Virus; 3, The Pathology of Yellow Fever; 4, Immunology; 5, The Arthropod Vectors of Yellow Fever; 6, The Mammalian Host in Yellow Fever; 7, The Clinical Aspects and Diagnosis of Yellow Fever; 8, Epidemiology; 9, Controlling Yellow Fever; 10, Costs and Man Power. Chapter 5 is one of the most interesting to the majority of *Mosquito News* readers for here is discussed the role of mosquitoes in the transmission of the disease; relationship between mosquitoes and the virus; relation of mosquito ecology to transmission of the fever; species of mosquitoes of interest in relation to the disease; 31 in South America, 20 in Africa, although fortunately not every one is susceptible to the disease; and arthropods other than mosquitoes with which sporadic studies were made. As Dr. Strode points out, "The comparatively vast scientific effort expended in laboratory and field work had its moments of sadness and drama. During the campaign 6 scientists on active duty succumbed to the disease." One of these, Theodore B. Hayne, and the writer spent many intriguing hours collecting and studying anophelines in the rice fields of Arkansas in 1921 under the supervision of Dr. M. A. Barber. Everyone interested in the biological sciences will find this book of great interest and value.—H. H. Stage.

The Distribution of Certain Liquids in the Esophageal Diverticula and Stomach of Mosquitoes. By Helen Louise Trembley. Amer. Journ. Trop. Med. and Hyg. 1(4):693–710. 1952. This is a carefully planned, unusually detailed, comparative study of the distribution of blood, 5% glucose or honey containing neutral red, and blood-glucose or blood-honey mixtures (9:1 in 0.85% saline) in the midgut and esophageal diverticula (dorsal sacs and crop) of more than 4,500 adult mosquitoes (4 species of *Anopheles*, 4 species of *Aedes*, and *Culex pipiens*), with special reference to mode of feeding.

Mosquitoes that obtained blood by biting man were allowed to feed continuously until satiated, were interrupted twice until satiated, or were allowed to feed until only partially full (removed while biting). Heparinized chick blood, glucose and honey, and mixtures of these were fed through *Baudruche* and chick skin membranes and from moistened cellucotton.

Blood taken by bite or by imbibing from cellucotton was generally found in the midgut and in one or more diverticula of all *Anopheles* tested (*An. quadrimaculatus, An. freeborni, An. albimanus, An. aztecus*), in only one of the four species of *Aedes, atropalpus,* and in *Culex pipiens*. In three out of four species of *Aedes* blood taken under similar conditions was distributed *only* to the midgut, confirming the work of Bishop and Gilchrist and of Lumsden who worked with *Ae. aegypti*.

When allowed to feed without interruption, distribution of blood in diverticula was found to vary according to species and was not a generic phenomenon: Trembley writes "Each of the eight species . . . would perhaps fall into its own niche, and with one exception . . . would differ significantly from all others. . . ." This species difference in blood distribution was not so pronounced when mosquitoes fed from cellucotton. Significantly fewer *Culex pipiens* had blood in diverticula after partial blood meals. In all other species, however, there was no significant difference in distribution.

Sugary solutions either through membranes or from cellucotton were primarily but not exclusively found in the diverticula of all species. Forty-four per cent of mosquitoes with sugar solution in diverticula also had some in midgut. However, the midgut was generally not so fully distended as the diverticula.

Interruption in biting did not produce statistically significant increases in numbers of mosquitoes with blood in diverticula. Interrupted feeding to satiation whether from arm or from cellucotton did *not* cause blood to be shunted to diverticula in *Ae. aegypti*. However, it is of great interest that when mosquitoes took only a *partial* blood meal by *continuous bite,* blood was found in the diverticula of all six species used (including *Ae. aegypti*) and this observation may partially explain the findings of Pawan and Fisk who found blood in the diverticula of *aegypti*. This observation also indicates that presence of blood in diverticula cannot be due to overdistention of the midgut.

Blood-glucose or blood-honey taken though membranes by *Ae. aegypti* passed primarily to the midgut. Trembley writes "There was a slight increase in the number of mosquitoes with blood in the diverticula when sugary solutions were added [to blood] . . .".

With the exception of *Culex pipiens,* there was

no regular sequence in filling of diverticula. In *C. pipiens* there was a tendency for blood to appear in the crop ahead of the dorsal diverticula.

Under the conditions used in this study (76° to 80° F.; R.H. 25 to 50%) 24 hours were required for emptying diverticula in *An. quadrimaculatus* and in *An. freeborni*. *C. pipiens* showed less tendency to empty diverticula in 24 hours. Trembley points out that peristalses are almost absent in dissected midguts of this species and hence might be responsible for such slow emptying.

Absence of mature ova did not ensure that blood would be dispatched entirely to the stomach.

The paper concludes with the pertinent warning that ". . . it is obvious that one cannot arbitrarily group these insects and refer to reactions or responses of 'mosquitoes'. This grouping, as well as insufficient numbers of mosquitoes observed, has been the outstanding weakness . . ." of much research on the complex study of the function of the diverticula in these insects.—Jack Colvard Jones.

STUDIES OF THE RESPONSES OF THE FEMALE *Aedes* MOSQUITO. PART IV. FIELD EXPERIMENTS ON CANADIAN SPECIES. Brown, A. W. A. Bull. Ent. Res. 42(2):575–582. 1951.

1. The responses of adult mosquitoes, consisting of several Canadian species of *Aedes*, were investigated under field conditions by employing heated and clothed robots.

2. Moisture increased the attractiveness of a warm body two to four times when the air temperature exceeded 60° F.; at cooler temperatures, surface moisture decreased the attractiveness.

3. Warmth increased the attractiveness of a body, so that a robot at 98° F. attracted three times as many mosquitoes as one at 50–65° F.

4. It may therefore be concluded that moisture is the major attractant factor when the air temperature exceeds 60° F., and warmth when the temperature is less than 60° F.

5. Carbon dioxide vapour emitted from an artificial head almost doubles the attractiveness of a warm body.

6. The vapours of ether and gasoline were significantly attractive. A sweat-soaked jerkin was more attractive than a water-soaked one.

7. Light colors were less attractive than dark ones, and luminescent dyes decreased the attractiveness of cloths. Green is less attractive than red or blue.

8. Glossy satins were less attractive than matt broadcloths. Greenish-khaki nylon cloth was much less attractive than khaki drill or cotton.

9. There was no evidence of interspecific differences in the responses of the mosquitoes investigated.—Author's summary.

ON THE DISTRIBUTION OF THE ANOPHELINE MOSQUITOES. (In Japanese.) Omori, Nanzaburo. Japanese J. Sanitary Zoology (Eisei Dobutsu). 1(1):1. 1950. (Pub. by the Japan Society of Sanitary Entomology, c/o Research Institute for Natural Resources, 4-chome, Hyakunin-cho, Shinjuku-ku, Tokyo, Japan. $2.00.)

Several kinds of anopheline larvae were found breeding in the forests of Thailand, scattered in numerous ponds and river pools, far distant from human dwellings. It was supposed by the author that the blood meal of the adult mosquitoes in that area came from wild animals, wandering in the forest. Breeding places of the following anophelines were mentioned: *A. minimus, A. maculatus, A. barbirostris, A. hyrcanus, A. annularis, A. aitkeni, A. insulaeflorum,* and *A. barbumbrosus.*—Toyohi Okada, Japan, and Don Pletsch. WHO, Taiwan.

ON MOSQUITOES OF HOKKAIDO. By Shogaki, Yukio. Jap. J. San. Zoology (Eisei Dobutsu). 1 (1):2–3. 1950. (For Publisher and price see preceding abstract.) (In Japanese.) 14 species of mosquitoes were collected from various parts of Hokkaido, including a *Theobaldia* form which may be a new species, and forms of *Aedes excrucians* and *Aedes cinereus* which may be new subspecies. *Aedes albopictus* and *Culex tritaeniorhynchus* were not found.—Okada and Pletsch.

THE EGGS OF JAPANESE *Anopheles* (REPORT I). (In Japanese.) By Otsuru, Masamitsu and Miyagawa, Mitsuo. Jap. J. San. Zoology (Eisei Dobutsu). 1(1):3–4. 1950. (For publisher and price, see first abstract of this series.) Obvious seasonal variations were observed in the eggs of *Anopheles hyrcanus* variety *sinensis*. From autumn to winter the eggs showed a progressive decrease in the breadth of the dorsal surface owing to the approach of the dorsal fringes toward one another, finally dividing the dorsal surface into two parts. Another type of seasonal variation was recognized in a series which seemed to be *Anopheles hyrcanus* var. *sinensis*. No distinct differences in egg characteristics were recognized between *A. koreicus* and *A. edwardsi*. Characteristics of the egg of *A. lindesaii* var. *japonicus* were also observed.—Okada and Pletsch.

A CONTRIBUTION TO THE KNOWLEDGE OF *Anopheles sintonioides* IN HAINAN ISLAND. (In Japanese.) By Niimura, Taro. Jap. J. San. Zoology (Eisei Dobutsu). 1(1):6–7. 1950. (For publisher and price, see first abstract of this series.) The larvae of *Anopheles sintonioides* together with *Megarhinus sp.* were found in small water reservoirs, usually under trees in graveyards on Hainan Island. *A. sintonioides* could be induced to bite man. Fourteen attempts were made to transmit malaria by the bites of this species, but results were negative. Adults were not found in light trap collections.—Okada and Pletsch.

ON THE SEASONAL DSTRIBUTION OF ANOPHELINE MOSQUITOES IN CENTRAL CHINA. (In Japanese).

By Mashiko, Kikuya. Jap. J. San. Zoology (Eisei Dobutsu). 1(1):10–12. 1950. (For publisher and price, see first abstract of this series.) The author found 4 types of seasonal variation of anopheline larvae in Central China: the *sinensis* type, *maculatus* type, *minimus* type, and *lindesayi* type. The *sinensis* type (e.g. *A. hyrcanus sinensis*) had 3 peaks in June, August and October, respectively, the August peak being the lowest. The *maculatus* type (e.g. *A. maculatus, A. pattoni*) showed a peak in midsummer and almost no appearance from October to June of the succeeding year. The *minimus* type (e.g. *A. minimus* and probably *A. jeyporiensis*) showed peaks in October and a minimum population in April-May; the larvae were seen all through the year. The *lindesayi* type (e.g. *A. lindesayi* and *A. aitkeni*) showed a minimum in August and also in April, the larvae being found throughout the year.—Okada and Pletsch.

On the Seasonal Prevalence of a Malaria Mosquito, *Anopheles hyrcanus sinensis* Wiedemann in Japan. (In Japanese). By Nomura, Kenichi. Jap. J. San Zoology (Eisei Dobutsu). 1(1):18–19. 1950. (For publisher and price, see first abstract of this series.) The total numbers of anopheline larvae inhabiting a rice field are considered proportional to the order of appearance of larvae, population, population density of larvae, and the area of the rice field. The first two factors were thought to be influenced by air temperature, water temperature and density of the rice plants.—Okada and Pletsch.

A Large Scale Experiment in Rice Field Mosquito Control. A. E. Whitehead. Ark. Agr. Expt. Sta. Rpt. Series 32, 15 pp. The test, conducted in Arkansas in 1951, included larval control on all rice grown on 22 sections of land, involving a total of almost 4,500 acres of rice. Two pounds of bentonite pellets containing 5 per cent of dieldrin to each acre of rice, were dispensed by an airplane. The material used and method of timing were based on three years of experiments by Prof. Whitehead and were reported in part at the March 1951 meeting of the American Mosquito Control Association in Chicago. Larval kills were 97% but only 77% of adult mosquitoes were killed.

A high proportion of the mosquitoes were *Psorophora confinnis* and *P. discolor*. Evidence from the experiment indicated an expenditure not exceeding $1 per acre. It was especially interesting to note that an experiment on such a large scale was made possible through contributions of materials by the American Colloid Company, Julius Hyman Co. and cash contributions by Monsanto Chemical Co., Arkansas Rice Growers Cooperative Association, Southern Rice Farms, Inc., Hartz Seed Co., Producers Rice Mill. First National Bank of Carlisle, Farm Bureau of De Witt, and Mr. M. H. Hopson, rice grower.—H. H. Stage.

Anopheles aztecus, Malaria, and Malaria Control in the Valley of Mexico. Downs, W. G. and Bordas, E. Journ. Nat. Malaria Soc. 10(4):350–358. 1951.

1. Malaria is endemic in the Xochimilco-Mixquic region of the Valley of Mexico at an altitude of 7,426 feet above sea level. *P. vivax* is the only species of endemic malaria. *A. aztecus* Hoffmann, 1935 has been found naturally infected in the region, can be readily infected in the laboratory, and has been shown to be able to transmit *P. vivax*. The species is commonly found in houses in the region and bites man freely. Basing judgment upon accumulated field and laboratory experience, it is considered as the sole malaria vector of importance in the region.

2. A malaria control program consisting of spraying of all houses and other buildings in the zones of the towns near the breeding areas, with DDT suspension made up from 50 per cent water-wettable powder, applied at the rate of 200 mgm. per square foot, has given, up to the present, excellent control of malaria. Parasite rates have dropped to zero and spleen rates have fallen markedly.

3. Control recommendations for the future are to re-spray after a two-year interval, and after that, to re-spray only when indicated by appearance of autochthonous cases of malaria.—Authors' summary.

Factors Influencing the Search for Anopheline Larvae in Sardinia. Trapido, H. Journ. Nat. Malaria Soc. 10(4):318–326. 1951. Field experience in Sardinia illustrates how uncertain the method of dipping for anopheline larvae is in establishing their presence or absence, when populations are very low. The premise that anopheline larvae are at or near the water surface is not always valid. Experiments are reported demonstrating the extent of surface avoidance following mechanical disturbance for the species *Anopheles claviger, A. hispaniola, A. algeriensis* and *A. l.labranchiae*. *Anopheles l.labranchiae* is shown to return to the surface more rapidly than the other three species. One minute after being disturbed, two per cent of the *labranchiae*, 39 per cent of the *claviger*, 36 per cent of the *hispaniola*, and 45 per cent of the *algeriensis* were still submerged. In 328 trials with *labranchiae* larvae the maximum submergence time was three minutes. The comparable figures for the other species were: *claviger*, 14 minutes in 300 trials; *hispaniola*, 35 minutes in 258 trials; *algeriensis*, 4 minutes in 31 trials.—Author's summary.

The Role of *Aedes leucocelaenus* in the Epidemiology of Jungle Yellow Fever in Brazil. Kumm, H. W. and Cerqueira, N. L. Bull. Ent. Res. 42(1):195–199. 1951. *Aedes leucocelaenus* transmits yellow fever virus readily in the laboratory and has twice been found infected in nature. Except in south Brazil this

species is much less common than *Haemagogus spegazzinii* among day-biting sylvan mosquitoes captured with human bait. *A. leucocelaenus* has a wide distribution throughout the forested area of this country. As a natural vector of jungle yellow fever in the northern part of Brazil it is of secondary importance when compared to *H. spegazzinii*.—Authors' summary.

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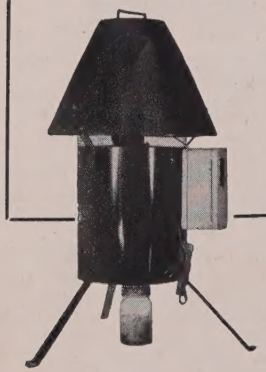

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